

METHODS OF ANALYSIS  
A. O. A. C.





# METHODS OF ANALYSIS, A.O.A.C., 5TH EDITION, 1940

## *Errata and Referees' Emendations\**

Page	Section.	
21	8(c)	Change " $\text{MgNO}_3$ " to "the $\text{Mg}(\text{NO}_3)_2$ soln."
32	42(d), line 3	Change "0.3061" to "0.30670."
34	47	Change "44" to "48."
36	54, par. 2, line 3	Change " $\text{H}_2\text{PO}_4$ " to " $\text{H}_3\text{PO}_4$ ."
37	58	Change last 3 lines to read as follows: "Continue as directed in XXVII, 60, beginning 'Add 0.3 g of $\text{KIO}_4$ for each 15 mg of Mn present'."
46	9, line 1	Change "31 g" to "13 g."
64	109, line 14	Transpose sentence "Test filtrate, etc." to end of sentence following, and change word "filtrate" to "distillate."
270	10, line 1	Change "1" to "2."
332	69	Add to title "Official, first action" and also the parenthetical statement "(Applicable to beverage concentrates.)"
349	Notes, 4th par., 7th line	Change " $\text{Na}_2\text{SO}_4$ " to " $\text{Na}_2\text{SO}_3$ ."
356	22, line 3	Change "2, 6, or 7" to "2 or 6."
357	23, line 1	Change "2, 6, or 7" to "2 or 6."
369	60, line 7	Place period after $\text{H}_2\text{O}$ and change word "and" to "Add."
371	64, Col. 2	Change "4.6" to "3.6."
386	54	Change "15" to "16."
416	46(h)	Delete this reagent.
433	29, 1st line under cut	Change "the standard" to "0.1 N."
442	47, line 8	Change "15" to "14."
504	46, 2nd equation	Change "8460" to "8640" and "9.51" to "95.1."
517	Ref. 26	Change "28" to "38."
585	78, line 10	Delete "on steam bath."
607	142	Change "Cincophen" to "Cinchophen."
608	146(c)	After word "of" add " $\text{KBrO}_3$ and 12 g of."
637	Ref. 102	Delete first 3 references and transpose first 2 to Ref. 104.
640	7, line 1	Change "media" to "medium."
667	6, last Col.	Change "31.4" to "41.4."
706	19, Col. 8	Change "00.00" to "100.00."
722	Col. 8	Change "0.05676" to "1.05676."
739	Basic phosphate slag, 6th line	Change "62 and 64 or 65" to "67 or 68."
740	Bromide in mineral water	Change "521" to "541."

Reprinted from *J. Assoc. Official Agr. Chemists*, February 15, 1942. See also report on "Changes in Methods" in the February number of each year.

OFFICIAL AND TENTATIVE

# METHODS OF ANALYSIS

OF THE

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

EDITORIAL BOARD

W. W. SKINNER, Chairman

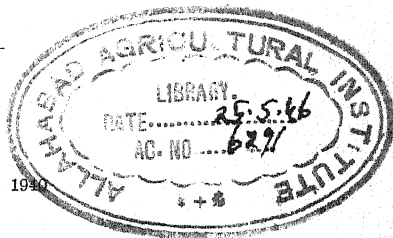
COMMITTEE ON EDITING METHODS OF ANALYSIS

E. M. BAILEY (Chairman), L. E. WARREN, J. W. SALE, G. G. FRARY,  
H. A. LEPPER and MARIAN E. LAPP

---

FIFTH EDITION, 1940

---



PUBLISHED BY THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

P.O. Box 540, BENJAMIN FRANKLIN STATION

WASHINGTON, D. C.

COPYRIGHT 1920, 1925, 1931, 1936, 1940 BY THE  
ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

The methods of the Association were also copyrighted in 1916, when they  
were published in the *Journal of the Association of*  
*Official Agricultural Chemists*

S-43  
W2760

Composed, Printed and Bound by  
The Collegiate Press  
George Banta Publishing Company  
Menasha, Wisconsin

## PREFACE TO FIFTH EDITION

---

In the introduction to the Second Edition of *Methods of Analysis*, A.O.A.C. prepared in 1924, in the fortieth year of the existence of the Association, Dr. Wiley said, "The most valuable contribution made to agriculture in the last forty years has been that of the standardization of the chemical and physical methods of research in agriculture by this Association." This is a challenge to the future from the past by one of the founders of our Association. The reply for 1940 is this Fifth Edition of this valuable book. The Association was organized in 1884 in response to an acutely felt need for standardization of the methods of analysis used in regulation and research. The first issue of these methods is a bulletin of fifty printed pages. The present volume of over 700 pages, therefore, is noteworthy evidence of the growth of the organization and the effort to meet the needs of its members and to satisfy a definite public demand. The small group of about twenty chemists that met in Philadelphia in 1884 could not have visualized the structure that has been built upon the foundations they then established and the assembling of over 500 persons at our annual convention in 1939. Our "*Book of Methods*" ranks as one of the most widely distributed publications of the world; truly, the sun never sets on its pages.

The expansion of the work of the Association has taxed the ingenuity of the editors to keep this edition to a convenient, handbook size that will prove most useful on the bench of the working chemist. To accomplish this purpose, of necessity something in form and style has been sacrificed to expediency. For instance, a saving of sufficient space was effected by the partial elimination of the articles "a", "an" and "the" to provide for the new material. Of the six chapters given in the Fourth Edition without text, material has been supplied for the following four: Fish and Other Marine Products, Vitamins, Microbiological Methods, and Microchemical Methods.

The Editorial Committee on the Fifth Edition is composed of E. M. Bailey (*Chairman*), L. E. Warren, J. W. Sale, G. G. Frary, H. A. Lepper, and Marian E. Lapp. To this committee the thanks of the Association are especially due.

As stated in the Preface to the Fourth Edition, our members should recognize that the strength of the Association rests upon the solid foundation of a system of critical investigation and practical tests of proposed

methods, enthusiastically and generously performed by the referees and associate referees. To all of these the Editorial Committee of 1940 extends its appreciation and thanks for their cordial and enthusiastic help.

W. W. SKINNER  
*President, Association of Official  
Agricultural Chemists*

Washington, D.C.  
July 1, 1940.

*Abstract from*

## PREFACE TO FOURTH EDITION

---

"The fourth issue of *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists* ('*Methods of Analysis*' or '*Book of Methods*') is presented to our members and to the public in accordance with the plan to publish a revision every five years. This book continues to grow in size and in the diversity of its subject-matter in harmony with the widening horizon of official chemists, who compose the Association. The philosophy of the Association, however, remains the same as in the beginning, and it is very aptly set forth by Dr. Harvey W. Wiley in the introduction to the first edition."

*Abstract from*

## PREFACE TO THIRD EDITION

---

"The third issue of '*Methods of Analysis*' or '*Book of Methods*', which are the abbreviated names of this publication, is offered to the members of the Association of Official Agricultural Chemists and to the public with a confidence that it will be as favorably received as were the previous editions. That this book of methods as originally conceived and executed fulfills the aims of its sponsors and meets the needs of a large group of official and control chemists is evidenced by the continued and unexpected demand which exhausted—six months before this edition was ready for distribution—the second edition of five thousand copies. The functions of the book and the philosophy of the development of the methods have been stated in the prefaces to former editions."

*Abstract from*

## PREFACE TO SECOND EDITION

---

"The methods of the Association of Official Agricultural Chemists are unique in several respects. They are the outgrowth of continual critical collaborative trial or test participated in by a large number of workers and undertaken in order to establish the accuracy of analytical results. They are subjected to a scrutiny of phraseology to insure clarity, probably unequaled in developing any similar methods. They are formulated solely by responsible Federal and State officials acting together and thus are based on underlying principles of equity. The Association has always encouraged to the utmost the cooperation of representatives of interested industries, but it has jealously reserved the final formulation of its methods to official chemists. Consequently, these methods have attained an enviable position in the fields of activity occupying the attention of the Association and are accepted as authoritative in matters at issue before the courts, both Federal and State."

*Abstract from*

## PREFACE TO FIRST EDITION

---

"In presenting this revision of the official and tentative methods of analysis of the Association of Official Agricultural Chemists, it is appropriate to give a brief statement of the organization of the Association, its purpose, and the procedure by which the methods are adopted.

"Membership in the Association is institutional and includes the State Departments of Agriculture, the State Agricultural Colleges and Experiment Stations, the Federal Department of Agriculture, and the Federal, State, and City offices charged with the enforcement of food, feed, drug, fertilizer, insecticide and fungicide control laws.

"The Association was founded at Philadelphia, Pa., September 9, 1884, by the following representative agricultural chemists of that time, the organization being the result of a series of informal meetings held the immediately preceding years:

Prof. H. W. Wiley, Chemist of the Department of Agriculture, Washington, D. C.

Mr. Clifford Richardson, Assistant Chemist of the Department of Agriculture, Washington, D. C.

Mr. Philip E. Chazal, State Chemist of South Carolina.

Dr. Chas. W. Dabney, Jr.,\* State Chemist of North Carolina.  
Dr. W. J. Gascoyne, State Chemist of Virginia.  
Dr. E. H. Jenkins, Connecticut Experiment Station.  
Prof. John A. Myers, State Chemist of Mississippi.  
Prof. H. C. White, State Chemist of Georgia.  
Mr. C. DeGhequier, Secretary National Fertilizer Association.  
Dr. Schumann, Dr. Lehmann, Mr. Gaines and others.”

\* Only surviving charter member.

# CONTENTS

	PAGE
Preface to Fifth Edition .....	v
Preface to Fourth Edition .....	vi
Preface to Third Edition .....	vi
Preface to Second Edition .....	vii
Preface to First Edition .....	vii
Illustrations .....	x
Definitions of Terms .....	xii
<b>CHAPTER</b>	
I. Soils .....	1
II. Fertilizers .....	20
III. * .....	
IV. Agricultural Liming Materials .....	41
V. * .....	
VI. Insecticides and Fungicides .....	44
VII. Caustic Poisons .....	80
VIII. Naval Stores .....	82
IX. Paints, Varnishes, and Constituent Materials .....	90
X. Leathers .....	116
XI. Tanning Materials .....	120
XII. Plants .....	125
XIII. Beverages (non-alcoholic) and Concentrates .....	146
XIV. Malt Beverages, Sirups and Extracts, and Brewing Materials ..	150
XV. Wines .....	164
XVI. Distilled Liquors .....	172
XVII. Baking Powders and Baking Chemicals .....	186
XVIII. Coffee and Tea .....	194
XIX. Cacao Bean and Its Products .....	201
XX. Cereal Foods .....	211
XXI. Coloring Matters in Foods .....	239
XXII. Dairy Products .....	269
XXIII. Eggs and Egg Products .....	308
XXIV. Fish and Other Marine Products .....	317
XXV. Flavoring Extracts .....	320
XXVI. Fruits and Fruit Products .....	335
XXVII. Grain and Stock Feeds .....	353
XXVIII. Meat and Meat Products .....	374
XXIX. Metals in Foods .....	390
XXX. Nuts and Nut Products .....	419
XXXI. Oils, Fats, and Waxes .....	423
XXXII. Preservatives and Artificial Sweeteners .....	454
XXXIII. Spices and Other Condiments .....	468
XXXIV. Sugars and Sugar Products .....	484
XXXV. Vegetables and Vegetable Products .....	518
XXXVI. Vitamins .....	524
XXXVII. Waters, Brine, and Salt .....	525
XXXVIII. Radioactivity .....	552
XXXIX. Drugs .....	559
XL. Microbiological Methods .....	639
XLI. Microchemical Methods .....	647
XLII. Standard Solutions .....	649
XLIII. Tables .....	654
Appendix, Definition of Terms, Fertilizers and Liming Materials .....	728
General Index .....	737

\* The subject matter of Chapters III, Sewage, and V, Agricultural Dust, may be studied by the Association later.



# ILLUSTRATIONS

	PAGE
Fig. 1. Quadruplicate Shaking Apparatus for Determination of Carbonate Carbon in Soils.....	2
Fig. 2. Absorption Tower.....	3
Fig. 3. Furnace Used in Determination of Iodine in Soils.....	7
Fig. 4. Apparatus for Determination of Selenium in Soils.....	11
Fig. 5. Steam Distillation Apparatus.....	15
Fig. 6. Apparatus for Automatic Filtration and Measurement of Lime Solutions.....	42
Fig. 7. Apparatus for Distillation of Arsenious Chloride.....	45
Fig. 8. Apparatus for Determination of Fluorine.....	51
Fig. 9. Dean and Stark Distilling Tube Receiver.....	62
Fig. 10. Apparatus Used in Volatilization Method for Determination of Mercury.....	72
Fig. 11. Transfer Loop and Manner of Using in Determination of Phenol Coefficient.....	77
Fig. 12. Articles Used in Grading Rosin.....	85
Fig. 13. Apparatus for Distillation of Turpentine Oil Over Open Flame.....	86
Fig. 14. Apparatus for Distillation of Turpentine Oil Over Bath.....	87
Fig. 15. Apparatus for Extracting Tanning Materials.....	124
Fig. 16. Suction Device Used in Micro Method for Determination of Calcium.....	128
Fig. 17. Apparatus for Determination of Iodine.....	137
Fig. 18. Apparatus for Determination of Lignin.....	144
Fig. 19. Absorption Buret.....	153
Fig. 20. Apparatus for Determination of Volatile Acidity.....	168
Fig. 21. Apparatus for Determination of Methyl Alcohol.....	179
Fig. 22. Apparatus for Gasometric Determination of Carbon Dioxide.....	187
Fig. 23. Graph Used in Correcting Cuprous Oxide for Effect of Sucrose.....	203
Fig. 24. Preparing the Dough for Panning.....	233
Fig. 25. Apparatus for Titration with Titanium Trichloride.....	259
Fig. 26. Apparatus for Determination of Melting Point.....	262
Fig. 27. Hortvet Cryoscope.....	275
Fig. 28. U. S. Bureau of Standards Tested Thermometer.....	278
Fig. 29. Photomicrographs Identifying Malted Milk and Allied Products.....	294
Fig. 30. Photomicrographs Identifying Malted Milk and Allied Products.....	296
Fig. 31. Mechanical Stirrer for Preparation of Butter Samples.....	298
Fig. 32. Apparatus for Drying Pentabromacetone by Aspiration.....	344
Fig. 33. Apparatus Used in Method II for Determination of Moisture.....	353
Fig. 34. Apparatus for Determination of Ammonia.....	375
Fig. 35. Van Slyke Apparatus for Determination of Amino Nitrogen.....	382
Fig. 36. Details of Deaminizing Bulb and Connection.....	383
Fig. 37. Generator to be Used with Gutzeit Method for Determination of Arsenic.....	391
Fig. 38. Distilling Apparatus for Determination of Arsenic by Bromate Method.....	393
Fig. 39. Apparatus for Determination of Lead by Electrolytic Method.....	400
Fig. 40. Apparatus for Determination of Melting Point.....	428
Fig. 41. Apparatus for Determination of Polenske Number.....	433
Fig. 42. Meloy Cottonseed Mixer and Divider.....	444
Fig. 43. Top View of Meloy Divider Showing Adjustment of Baffles.....	445
Fig. 44. Roller-type Experimental Flouring Mill Suitable for Grinding Flaxseed Samples.....	448
Fig. 45. Dipping-type Refractometer with Interchangeable Double Prism Heads Suitable for Refractometric Determination of Oil Content of Flaxseed.....	449
Fig. 46. Monier-Williams Apparatus for Determination of Sulfurous Acid.....	464
Fig. 47. Apparatus for Determination of Formic Acid.....	466
Fig. 48. Apparatus for Determination of Volatile Oil.....	470

Fig. 49. Types of Oil Separatory Traps.....	471
Fig. 50. Reaction Cylinder to be Used in Determination of Bromide.....	548
Fig. 51. Apparatus for Determination of Radioactivity by Emanation Method.....	553
Fig. 52. Gamma Ray Electroscope.....	557
Fig. 53. Scrubber Trap for Ammonia Distillation.....	578
Fig. 54. Apparatus Used in Determination of Terpin Hydrate and Codeine in Elixir.....	580
Fig. 55. Automatic Percolator.....	581
Fig. 56. Mojonnier-Type Extraction Tube.....	582
Fig. 57. Continuous Extraction Apparatus.....	594
Fig. 58. Apparatus for Determination of Ether.....	606
Fig. 59. Fitted Flask for Determination of Nicotinic Acid.....	611
Fig. 60. Apparatus for Determination of Arsenic in Iron-Arsenic Tablets....	615
Fig. 61. Semi-micro Zeisel Methoxyl Apparatus.....	647

## DEFINITIONS OF TERMS AND EXPLANATORY NOTES

---

- (1) The term "water" means distilled water.
- (2) The term "alcohol" means 95% alcohol.
- (3) The reagents listed below, unless otherwise specified, have the approximate strength stated and conform in purity with the requirements of the United States Pharmacopoeia.

	<i>Specific gravity</i>
Sulfuric acid.....	1.84
Hydrochloric acid.....	1.184
Nitric acid.....	1.42
Fuming nitric acid.....	1.50
Glacial acetic acid.....	1.048 (25°)
Hydrobromic acid.....	1.38
Ammonium hydroxide.....	0.90
Phosphoric acid.....	85% conc. by weight

(4) All other reagents and test solutions, unless otherwise described in the text, conform to the requirements of the United States Pharmacopoeia or of the American Chemical Society. When the anhydrous salt is intended, it is so stated; otherwise the salt referred to is the crystallized product.

(5) In the expressions (1+2), (5+4), etc., used in connection with the name of a reagent, the first numeral indicates the volume of the reagent used, and the second numeral indicates the volume of water. For example, hydrochloric acid (1+2) means a reagent prepared by mixing one volume of hydrochloric acid with two volumes of water. When one of the reagents is a solid, the expression means parts by weight, the first numeral representing the solid reagent and the second numeral the water.

(6) In making up solutions of definite percentage it is understood that  $x$  grams of substance is dissolved in water and made up to 100 ml. Although not theoretically correct, this procedure will not result in any appreciable error in any of the methods given in this book.

(7) For the sake of simplicity the abbreviations Cl and I instead of  $\text{Cl}_2$  and  $\text{I}_2$  are used for chlorine and iodine. Similar abbreviations have been used in other cases.

(8) All calculations are based on the table of international atomic weights, Table I, under XLIII.

(9) Unless otherwise indicated all temperatures are expressed as degrees Centigrade.

(10) Directions for standardizing reagents are given in Chapter XLII.

(11) To conserve space, the abbreviation "ca" is used for "approximately" or "about," and most of the articles have been eliminated.

# Official and Tentative Methods of Analysis

OF THE

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

### I. SOILS<sup>1</sup>—TENTATIVE

1

#### DIRECTIONS FOR SAMPLING

(In view of the variability of soils, it seems impossible to devise an entirely satisfactory method for sampling. It is obvious that details of procedure should be determined by the purpose for which the sample is taken.)

Remove from surface all vegetable material not incorporated with soil. Take sufficient number of samples to insure a composite sample that will be representative of the tract sampled, to average depth of plowed soil, usually about 7", and also take composite sample from each important and distinctly different soil stratum to depth of 40", using soil tube or auger. In using soil auger, enlarge first boring before boring below plowed depth and carefully clean out hole to prevent contamination of successive sub-strata while withdrawing samples. Obtain samples when soil is reasonably dry. Thoroughly mix samples of each depth and dry in a well ventilated, cool place.

To calculate percentage results obtained by analysis to pounds per given area of soil, determine weight of a given volume of soil as it lies in field.

2

#### PREPARATION OF SAMPLE

(a) Crush any soil lumps in air-dried soil by rubbing in porcelain mortar or by any other equally effective method that will not crush rock fragments, and pass thru sieve having circular openings 0.5 mm in diameter. Thoroughly mix sifted material and preserve in suitable stoppered container. Weigh, and discard detritus.

(b) If necessary for determination of total quantity of any constituent, *pulverize* more finely a sub-sample of (a).

Record any deviations from this procedure.

3

#### MOISTURE

Place 2 g of prepared sample, 2(a), in wide-mouthed weighing bottle and heat at 105° in electric oven for 5 hours. Report loss in weight as percentage of moisture-free weight of sample.

4

#### LOSS ON IGNITION

(This method only approximates the organic matter content, especially for soils containing much combined water.)

Ignite soil from 3 to full redness in Pt dish, stirring occasionally, until organic matter is destroyed. Cool in desiccator, weigh, and report percentage loss in weight as "loss on ignition." Utilize residue for 11.

## CARBONATE CARBON

## APPARATUS

*Quadruplicate shaking apparatus for evolution of carbonate carbon in soils of high or low carbon content.*—Apparatus (Figs. 1 and 2) consists of horizontal holder (*H*) 21" long,  $\frac{7}{8}$ " thick, and  $1\frac{3}{4}$ " wide, having properly spaced slots made to fit loosely the neck of a 300 ml Erlenmeyer flask taking a No. 6 rubber stopper. The holder

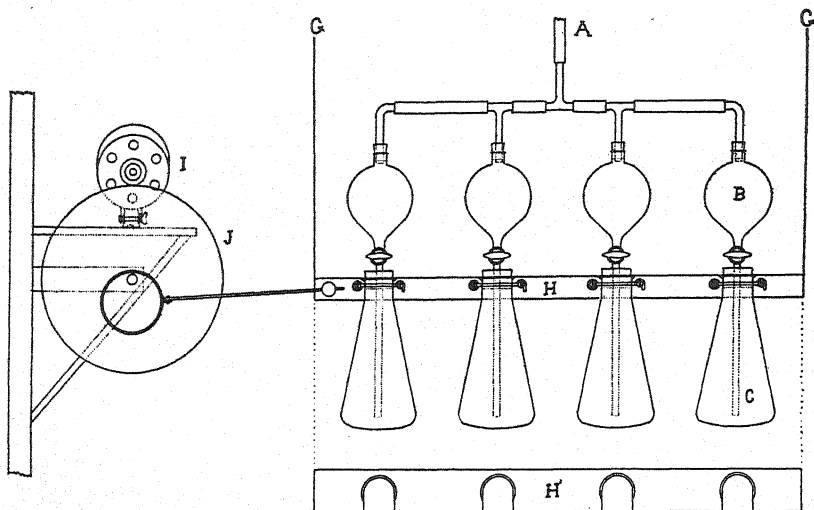


FIG. 1.—QUADRUPLICATE SHAKING APPARATUS FOR DETERMINATION OF CARBONATE CARBON IN SOILS

is suspended horizontally from a bar by means of brass strips  $1\frac{3}{4}$ " wide and 24" long. The common intake for purification of incoming air leads from a tube about 25" long.

This tube stands upright, extending thru rubber stopper in 1 liter Erlenmeyer flask, and has inserted in top a large N distillation bulb to prevent mechanical carrying over of any of purifying NaOH.

Driving wheel (*J*) is  $\frac{1}{2}$ " thick and 7" in diameter. Eccentric attached to its face is  $\frac{1}{2}$ " in thickness and 2" in diameter, and is grooved to permit free rotation of driving shaft, which is fastened to end of holder by means of a binding post. Power for agitation is supplied by motor (*I*), a sewing machine, or small desk fan motor. If motor available has no rheostat, its speed can be easily controlled by battery of 4 lamps. Motor is hinged upright on the support so that pulley will rest upon edge of driving wheel. To reduce noise the pulley of motor is inserted into rubber stopper. Or driving wheel may be made to carry a belt that is driven by pulley of small motor.

Absorption tower (*D*) is at least 25" high and 1" in diameter. It contains alternating pockets of solid glass rods and small glass beads resting upon an inverted test tube  $2\frac{3}{4}$ " long. The rubber connection on intake cock of tower is used to disconnect the glass tube that extends to rubber connection on safety bulb tube leading from flask *C*.

## DETERMINATION

6

I. Volumetric Method<sup>2</sup>

Pulverize sample to pass 60-mesh sieve, so as to expose fully to action of liberating acid any calcite that may be included in quartz crystals. For soils low in carbonates use 10, 25, or 50 g charge in quadruplicate shaking device, 5.

Introduce charge into 300 ml evolution flask (C, Figs. 1 and 2), and aspirate 5 min. to free apparatus of atmospheric  $\text{CO}_2$ ; release suction, and introduce 10, 25, or 50 ml of 0.5 N NaOH or KOH soln into absorption tower. Apply a suction of 5" and introduce 60 ml of HCl (1+9) containing 5% of  $\text{SnCl}_2$  upon soil contained in Erlenmeyer flask, regulating intake of air by means of a screw cock placed just beyond absorption tower. Agitate and aspirate 60 min. at rate of 3-4 bubbles per second. Then release suction and draw off absorbent soln into 500 ml flask, washing tower with a succession of fillings of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  to volume of 450 ml. Add 10 ml of neutral aqueous soln of  $\text{BaCl}_2$  (250 g of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  per liter), make to volume, agitate, and allow to stand 4 hours. Titrate excess of hydroxide, using phenolphthalein indicator. With small-bore buret permitting split-drop readings to hundredths of a ml, use 0.5 N acid for titration; with burets of larger bore, use 0.1 or 0.25 N acid. Report result as percentage of carbonate C or  $\text{CO}_2$ .

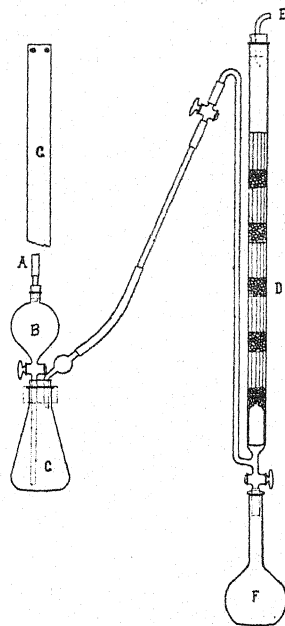


FIG. 2.—ABSORPTION TOWER

7

II. Gravimetric Method<sup>3</sup>

Proceed as in 6, but in lieu of the NaOH and KOH absorbent use absorption tube filled with ascarite and preceded in train by tubes containing an  $\text{Ag}_2\text{SO}_4$  suspension in  $\text{H}_2\text{SO}_4$  (1+19),  $\text{H}_2\text{SO}_4$ , and anhydrous, in order. Report increase in weight found for ascarite tube in percentage of carbonate C or  $\text{CO}_2$ .

NOTE.—Special consideration should be given to soils that have been treated with magnesite or dolomite or those known to be derived from the limited magnesite area, or from the glaciated region where transported dolomite may occur in considerable quantities. For such soils, agitate the HCl- $\text{SnCl}_2$ -soil suspension until  $\text{CO}_2$  evolution has subsided. Then apply heat to agitated suspension, with provision for condensation, until no  $\text{CO}_2$  evolution is indicated by the liquids in absorption train. Remove heat, discontinue agitation, and draw  $\text{CO}_2$ -free air thru apparatus 20 min. The absorption may be accomplished by either volumetric (6) or gravimetric (7) procedure.

ORGANIC CARBON<sup>4</sup>

8

## APPARATUS

- (a) *Oxygen cylinder*.—With pressure-regulating valve.
- (b) *Electric combustion furnace*.—With rheostat, and with  $\frac{7}{8} \times 24$ " fused silica

tube containing an 8" loosely packed core of platinized asbestos. (CuO may be used if temp. of 950° is not exceeded.)

(c) *Purification and absorption train.*—Place 2 scrubber bottles containing 10% soln of KOH, followed by Hg valve, between O supply and intake end of furnace. Provide an asbestos-filled Cu coil with handle as insulating plug on intake end of combustion tube; also use asbestos plug to insulate rubber stopper at outlet end of combustion tube. The outcoming current is dried and purified by an H<sub>2</sub>SO<sub>4</sub> scrubber, tube containing 40-mesh granulated Zn, and tube of P<sub>2</sub>O<sub>5</sub>, or equivalent drying material, in order. Connect drying tube with Nesbit or similar absorption tube filled with alternate layers of glass wool and ascarite and protect against moisture and back pressure at its outlet by a Fisher bubble counter containing H<sub>2</sub>SO<sub>4</sub>.

9

## DETERMINATION

Bring furnace to 900–950°. Connect train and sweep out apparatus by adjusted flow of O. Weigh absorption tube against a counterpoise, replace it in train, and introduce well within heated zone an alundum boat containing 2 g charge of soil admixed with 2 g of finely divided CuO. Close intake and open Nesbit bulb. When no more gas passes thru absorption train, connect it with suction, admit a flow of O, and aspirate 30 min. Close Nesbit tube, disconnect, and weigh against counterpoise. Correct total evolution of CO<sub>2</sub> for carbonate CO<sub>2</sub> (6 or 7) and report as organic C, or CO<sub>2</sub>. (There should be no carbonates in residue in case of acid, calcareous, or dolomitic soils.) Correct unleached alkali soils for original and any residual CO<sub>2</sub>.

10

## TOTAL NITROGEN

Digest 10 g of soil in a 500 ml Kjeldahl boiling flask with 30–40 ml of H<sub>2</sub>SO<sub>4</sub> and ca 10 g of salt mixture composed of 10 parts of K<sub>2</sub>SO<sub>4</sub> or anhydrous Na<sub>2</sub>SO<sub>4</sub>, 1 part of FeSO<sub>4</sub>, and  $\frac{1}{2}$  part of CuSO<sub>4</sub>. Continue digestion until mixture is colorless or nearly so. After cooling, dilute contents of flask with H<sub>2</sub>O, add excess of an approximately 45% NaOH soln, connect flask with condenser, and distil 150 ml into standard acid as directed under II, 21. (Distillation may be carried out in digestion flask, or, if preferred, soln may be transferred to an Armsby Cu pot.) Titrate excess of acid with 0.1 N or N/14 alkali, using methyl red or cochineal indicator, II, 19(h) and (i). Report as percentage of N.

11

SODIUM CARBONATE FUSION<sup>5</sup>

Mix residue from 4, ground to 100-mesh, with 10 g of Na<sub>2</sub>CO<sub>3</sub> in 30 ml Pt crucible. Cover crucible and heat at low redness until fusion begins; increase heat to a clear, quiet fusion; then give full heat of Meker burner 20 min., with flame oblique. Cool the melt, place in 250 ml porcelain evaporating dish, add 100 ml of H<sub>2</sub>O, and digest to disintegration on water bath. Cover dish, add 50 ml. of HCl, digest 15 min., and wash cover. Evaporate to dryness and bake 2 hours at 110° (or substitute HClO<sub>4</sub> for HCl, and thereby obviate baking).

12

## SILICA

Take up the residue from 11 in HCl (1+9) and filter mixture so obtained. Wash with hot H<sub>2</sub>O containing 5 ml of HCl per liter. Collect filtrate and washings in dish, preferably a casserole, and dehydrate on steam bath until the SiO<sub>2</sub> assumes a crystalline appearance. Moisten with HCl and repeat dehydration 2 hours. Add 5 ml of

HCl and 100 ml of hot  $\text{H}_2\text{O}$ , mix thoroly, filter, and wash with hot  $\text{H}_2\text{O}$  containing 5 ml of HCl per liter. Add residue to main portion of  $\text{SiO}_2$  obtained from first filtration. Make up combined filtrate and washings to 500 ml and use in subsequent determinations. (For soils of low Ca content, use the entire filtrate.) Place the two  $\text{SiO}_2$  residues with filters in a Pt crucible. Moisten with saturated  $\text{NH}_4\text{NO}_3$  soln. Ignite with low heat at first to burn off filter paper and then with strong flame, preferably a blast lamp, to constant weight; cool in desiccator and weigh. Report as percentage of  $\text{SiO}_2$ .

### 13 OXIDES OF IRON, ALUMINUM, PHOSPHORUS, AND TITANIUM

To 250 ml aliquot of 12, add 10 ml of HCl and few drops of methyl red indicator; heat to gentle boiling and add  $\text{NH}_4\text{OH}$  (1+1) until precipitate forms and the indicator in soln just changes to a distinct yellow. Boil not longer than 2 min. and filter rapidly. Wash precipitate 6–8 times with hot 2%  $\text{NH}_4\text{NO}_3$  soln. Return precipitate and filter to original beaker, add 10 ml of HCl, and macerate filter with policeman. Dilute with  $\text{H}_2\text{O}$ , heat to dissolve precipitate, dilute to ca 200 ml, and reprecipitate as directed above. Wash thoroly with hot  $\text{NH}_4\text{NO}_3$  soln until free of chlorides. Combine first and second filtrates and save for Ca and Mg determinations.

Place precipitate in Pt crucible; dry, and ignite over low flame of Bunsen burner until C has been oxidized. Heat to bright redness ca 10 min. Cool in desiccator and weigh in covered crucible as  $\text{Fe}_2\text{O}_3$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{P}_2\text{O}_5$ , and  $\text{TiO}_2$ .

### 14 CALCIUM

Concentrate combined filtrates and washings from 13 to ca 50 ml; make slightly alkaline with  $\text{NH}_4\text{OH}$  (1+1); and add, while still hot, saturated  $\text{NH}_4$  oxalate soln dropwise as long as any precipitate is produced, and then an excess sufficient to convert the Mg salts also into oxalate. Heat to boiling, allow to stand 3 hours or longer, decant clear soln thru filter, pour 15–20 ml of hot  $\text{H}_2\text{O}$  on precipitate, and again decant clear soln thru filter. Dissolve any precipitate remaining on filter by washing with hot HCl (1+9) into original beaker, wash 6 times with hot  $\text{H}_2\text{O}$ , and then reprecipitate, boiling hot, by adding  $\text{NH}_4\text{OH}$  and a little  $\text{NH}_4$  oxalate soln. Allow to stand as before and filter thru same filter. Wash free from chlorides with hot  $\text{H}_2\text{O}$ . Reserve filtrates and washings from both precipitations for determination of Mg, 16. Complete determination by one of following procedures and report as percentage of  $\text{CaO}$ .

(a) Ignite precipitate in a crucible either over an S-free blast or in electric oven at  $950^\circ$  to constant weight, cool in desiccator, and weigh  $\text{CaO}$ .

(b) Incinerate filter over low flame, mix ignited precipitate with finely pulverized and dried mixture of equal parts of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NH}_4\text{Cl}$ , and drive off excess of sulfate by careful heating of upper portion of crucible. Complete ignition, cool in desiccator, and weigh the  $\text{CaSO}_4$ .<sup>6</sup>

(c) Perforate apex of cone; wash Ca oxalate precipitate into beaker used for precipitation and then wash filter with hot  $\text{H}_2\text{SO}_4$  (1+4), and titrate hot ( $85$ – $90^\circ$ ) with 0.1 N  $\text{KMnO}_4$ .

### MAGNESIUM

#### 15

#### REAGENT

*Phosphate soln.*—Dissolve 100 g of  $(\text{NH}_4)_2\text{HPO}_4$  or  $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$  in hot  $\text{H}_2\text{O}$ , cool, dilute to 1 liter, and introduce 5 ml of  $\text{CHCl}_3$ .



16

## DETERMINATION

To combined filtrates and washings, 14, add 2 ml of *M*/1 citric acid, 100 ml of  $\text{NH}_4\text{OH}$ , and 50 ml of alcohol. Then add with constant stirring 25 ml of the phosphate soln and let stand 12–24 hours. Filter, wash twice with  $\text{NH}_4\text{OH}$  (1+9), and dissolve precipitate in  $\text{HNO}_3$  (1+4), washing soln into original beaker to volume of 100–150 ml. To this soln add  $\frac{1}{5}$  volume of  $\text{NH}_4\text{OH}$  and 2 drops of the phosphate soln. Stir vigorously and allow to stand 3 hours or longer. Filter thru a Gooch crucible, wash with the  $\text{NH}_4\text{OH}$ , moisten filter with saturated ammoniacal soln of  $\text{NH}_4\text{NO}_3$ , ignite, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Report as percentage of  $\text{MgO}$ . Correct weight of  $\text{Mg}_2\text{P}_2\text{O}_7$  for occluded  $\text{Mn}_2\text{P}_2\text{O}_7$  as directed in XXXVII, 73.

17

## MANGANESE

Treat 1 g of 100-mesh soil with 5 ml of  $\text{HF}$  and 5 ml of  $\text{H}_2\text{SO}_4$  (1+1); evaporate and heat gently to dryness. Repeat addition of  $\text{HF}$  until all silicates are decomposed. Dissolve in  $\text{H}_2\text{O}$ , add  $\text{HNO}_3$ , and evaporate to dryness. Again dissolve in  $\text{H}_2\text{O}$ , add 25 ml of  $\text{HNO}_3$  (1+2) and ca 0.5 g of Na bismuthate, and heat until permanganate color disappears. Proceed as directed under XXXVII, 75, beginning, "Add a few drops of a 10% soln of  $\text{NH}_4$  bisulfite or saturated Na bisulfite to clear soln." Report as percentage of  $\text{Mn}_2\text{O}_3$ .

IODINE<sup>3</sup>

18

*Fusion Method*

Place in a clean 400 ml iron crucible 5 g of air-dried soil, ground to pass a 100-mesh sieve, 10 g of I-free  $\text{KOH}$  pellets, and 5 ml of  $\text{H}_2\text{O}$ , and stir with clean piece of No. 6 iron wire until most of pellets have dissolved. Place crucible in 4.5" Bunsen tripod and heat moderately with flame of burner, stirring contents of crucible rapidly until  $\text{H}_2\text{O}$  has been driven off and dry granular fused mass remains. Avoid heating crucible to redness after  $\text{H}_2\text{O}$  has been expelled. Cool crucible and add ca 50 ml of  $\text{H}_2\text{O}$  and allow it to stand, with occasional stirring, until the fused mass has slaked to a sludge. Transfer contents of crucible to 500 ml beaker, police, and wash inside walls of crucible thoroly. Add small strip of litmus paper, ca 0.1 g of K bisulfite, and  $\text{HCl}$  (1+1), stirring until contents of beaker have acid reaction and distinct odor of  $\text{SO}_2$  can be detected. Add saturated soln of  $\text{K}_2\text{CO}_3$  from a short-stemmed pipet, stirring until entire mass has an alkaline reaction. Pour precipitate of silica, Fe and Al hydroxides onto folded filter and wash thoroly by addition of ca 25 ml portions of hot  $\text{H}_2\text{O}$  at a time, allowing each portion to drain thru before adding another. (Volume of filtrate and washings should be ca 500 ml.) Transfer filtrate to porcelain dish and evaporate until a sludge of salts remains. (A small current of compressed air directed on surface of soln during heating on water bath will hasten evaporation.) Remove dish from water bath and add 50 ml of pure alcohol to the hot sludge of salts. Stir thoroly with a policeman until dish has attained room temp. (The salts assume a pasty consistency with much stirring while they are hot and this condition facilitates soln of the KI in the alcohol.) Decant the alcoholic extract thru small folded filter into beaker, further extract residue with one 25 ml and one 10 ml portion of alcohol, and decant thru filter into beaker containing first alcoholic extract. Dissolve salts adhering to filter paper and those in dish in hot  $\text{H}_2\text{O}$ , and evaporate soln to a sludge. Extract sludge with one 25 ml and two 10 ml portions of alcohol, as directed in treatment of first sludge of salts, and repeat process of extraction, using three 10 ml portions of alcohol. Combine alcoholic extracts and evaporate to dryness. Dissolve residue in small quantity of hot

H<sub>2</sub>O, rinse into 250 ml beaker, and evaporate to dryness. Dissolve residue in few drops of hot H<sub>2</sub>O and a drop of saturated soln of K<sub>2</sub>CO<sub>3</sub>, and add 10 ml of alcohol. Stir precipitated salts rapidly with glass rod ca 10 min., and decant alcoholic extract thru small filter into 150 ml beaker. Further stir residue, extract with two 5 ml portions of alcohol, decant thru filter into beaker, rinsing filter with a few ml of alcohol, and evaporate extract to dryness. Dissolve residue, consisting of ca 0.05 g, in few drops of hot H<sub>2</sub>O and rinse into 25 ml Pt or Sillimanite dish, evaporate to dryness, and dry at 100° 1 hour. Heat dish at ca 400° in electric furnace having pyrometer attachment until organic matter is burned or charred so that it will not give a turbid soln when H<sub>2</sub>O is added. If turbid soln is obtained, evaporate to dryness and burn again. After cooling dish, dissolve residue in few drops of H<sub>2</sub>O, at

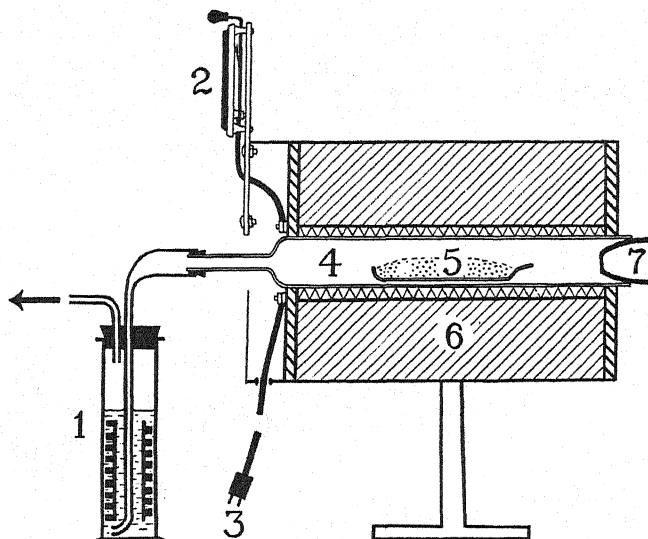


FIG. 3.—FURNACE USED IN DETERMINATION OF IODINE IN SOILS

1—Gas wash bottle, 2—Rheostat, 3—Power line, 220 volts, 4—Quartz tube, 5—Sample (in Sillimanite boat), 6—Electric tube furnace, 7—Stopper (alundum crucible).

room temp., filter soln, and wash into 30 ml separatory funnel. (Soln should be colorless, slightly alkaline, and have volume of ca 5 ml). Make slightly acid with a few drops of H<sub>2</sub>SO<sub>4</sub> (1 + 1), add ca 0.01 g of pure K bisulfite to separatory funnel, stopper, and shake a few seconds to reduce any iodate to iodide. Remove stopper, and add 1 ml of pure CS<sub>2</sub> and ca 2 ml of 10% soln of pure I-free K or NaNO<sub>2</sub>. Stopper and shake separator vigorously ca 1 min. Place separator in stand and allow the CS<sub>2</sub> containing the I to collect and settle ca 5 min. If the CS<sub>2</sub> is light pink in color, it contains all the I; if it is a deep pink, run it carefully into centrifuge tube and further extract soln in separator with 1 ml portions of CS<sub>2</sub> until last portion is only slightly pink. Combine CS<sub>2</sub> extracts and centrifuge. Place a portion of clear extract in micro cup of colorimeter and compare quickly with freshly prepared I standard having comparable depth of color. Report results in p.p.m.

19 *Volatilization Method by Heating Soil in an Electric Tube Furnace*

Place in porcelain boat 25–100 g of soil ground to pass 2-mm sieve and insert boat in silica tube, Fig. 3. Use 2 wash bottles (Milligan), each containing 100 ml of 2.5% soln of  $K_2CO_3$ . Connect first wash bottle with silica tube by means of a glass thimble made to fit a rubber gasket on small end of combustion tube. Connect wash bottles closely with rubber tubing. Attach the last wash bottle to suction pump regulated to draw vapors at a moderate rate into the wash bottles during time the soil is heated. (About 1 hour is required to attain maximum temp. of furnace, which is maintained ca 2 hours.) Disconnect wash bottles, rinse soln into porcelain dish, and evaporate to dryness. Dissolve residue in few drops of hot  $H_2O$ , rinse into 150 ml beaker, and evaporate until ca 2 ml remains. Add to beaker 10 ml of pure alcohol, stir rapidly with glass rod ca 10 min., and decant extract thru small filter into 150 ml beaker. Extract residue with two 5 ml portions of alcohol and decant thru filter into beaker. Evaporate alcohol and wash residue into 25 ml Sillimanite dish; evaporate to dryness, dry at  $100^\circ$ , and heat at ca  $400^\circ$  for 10 min. in electric furnace having pyrometer attachment. Remove dish from furnace; cool, and dissolve residue in few drops of cold  $H_2O$ . Filter, and wash into 30 ml separator. Liberate, absorb, and determine I as directed in 18.

## SULFUR\*

## 20

## PREPARATION OF SOLUTION

Weigh 5–10 g of the soil, 2(a), prepared to pass 0.5 mm sieve, into 100 ml Ni crucible; add equal weight of anhydrous  $Na_2CO_3$ ; and mix well with stout Ni stirring rod of such length as to permit introduction into furnace to be used in fusion. Pipet carefully 4 ml of  $H_2O$  into each 10 g of soil; stir well to stiff paste, adding more  $H_2O$  if necessary, dropwise. Immediately add successive portions of ca 1 g of S-free  $Na_2O_2$ , stirring well after each addition to obviate excessive frothing and overflow. Continue to add peroxide until mixture becomes dry and granular, and add, as a surface coating, enough to make total peroxide addition 25 g per 10 g of soil. Place mixture in electric furnace; maintain temp. at  $400$ – $500^\circ$  during first half hour, then raise rapidly to bright red heat (ca  $900^\circ$ ); and continue fusion at this temp. ca 10 min. Withdraw crucible from muffle, quickly manipulate so as to cause melt to spread out in thin sheet over interior of crucible, and cool rapidly by contact with some good conductor in cool atmosphere. Place chilled crucible sidewise in 600 ml beaker and cover with  $H_2O$ . Add ca 5 ml of alcohol to decompose the Na manganate. Cover beaker with watch-glass, place on cold hot plate, and apply heat. Boil briskly until melt is disintegrated (30 min.). When suspension has assumed a flesh-colored, flocculent appearance, with no glassy green lumps in interior of crucible, remove crucible and rod from beaker and wash any flaky particles back into beaker with a policeman, rinsing several times with hot  $H_2O$ . (If small glassy particles still cling to inside of crucible, disintegrate by boiling  $H_2O$  over hot plate or small flame and add to main portion.) Filter immediately by suction thru 9 cm Büchner funnel into liter beaker placed under bell jar. When no more liquid can be drawn thru filter, return residue, together with filter paper, to original beaker, washing any adhering particles carefully from funnel. Add ca 1 g of  $Na_2CO_3$ , macerate with the policeman, add 75–100 ml of  $H_2O$ , and bring to brisk boil while stirring vigorously. Again filter thru Büchner funnel, using suction, until nearly dry, and wash with 20 ml portions of hot  $H_2O$  to total volume of 500 or 700 ml for the 5 or 10 g charge, respectively.

21

## DETERMINATION

Add from buret slowly and with stirring, sufficient HCl to neutralize the soln, using methyl red indicator, II, 19(i). Add 0.5 ml excess of the HCl and concentrate by heating to volume of 400 ml. If cloudiness appears, it is imperative to remove  $\text{SiO}_2$  by evaporation and dehydration below  $120^\circ$ . If soln is perfectly clear, heat to boiling, add slowly 10 ml of 5%  $\text{BaCl}_2$  soln, and allow to stand overnight. Filter on dense filter paper, place paper in Pt crucible, and ignite in electric furnace. Cool in desiccator, and weigh as  $\text{BaSO}_4$ . To insure against possible inclusion of  $\text{SiO}_2$ , add 2 drops of HF and 1 drop of  $\text{H}_2\text{SO}_4$  (1+1), heat carefully, reignite, and weigh. Report as percentage of S or  $\text{SO}_3$ .

## PHOSPHORUS

22

*Sodium Peroxide Method*

Place 10 g of  $\text{Na}_2\text{O}_2$  in either iron or porcelain crucible and thoroly mix with it 5 g of the prepared soil, 2(a). If soil has a low organic matter content, add a little starch to hasten reaction. Heat mixture carefully by applying flame of Bunsen burner directly upon surface of charge and sides of crucible until reaction starts; cover crucible and keep at low red heat 30 min. Do not allow fusion. By means of large funnel and stream of hot  $\text{H}_2\text{O}$  transfer charge to 500 ml volumetric flask, acidify with HCl, and boil. Cool, and make to mark. (If reaction has taken place properly, there should be no particles of undecomposed soil in bottom of flask.) Allow the  $\text{SiO}_2$  to settle and draw off 200 ml of clear soln.

Or, in lieu of above, oxidize 5–10 g of the material and disintegrate melt as directed under 20. Dissolve residue with  $\text{HNO}_3$  (1+1), dilute to 500 ml, and withdraw 200 ml aliquot.

Precipitate the Fe, Al, and P with  $\text{NH}_4\text{OH}$  (1+1), filter, wash several times with hot  $\text{H}_2\text{O}$ ; return precipitate to beaker, and dissolve in hot HCl (1+4), pouring the acid upon filter to dissolve any precipitate remaining. Evaporate soln and washings to complete dryness on water bath. Take up with  $\text{HNO}_3$  (1+4), heating if necessary, and filter to remove  $\text{SiO}_2$ . Evaporate filtrate and washings to ca 10 ml and add 2 ml of  $\text{HNO}_3$ . Neutralize excess of acid with  $\text{NH}_4\text{OH}$  (1+1) and add  $\text{HNO}_3$  until soln becomes clear, avoiding an excess. Heat to  $40\text{--}50^\circ$  in water bath, add 15 ml of molybdate soln, II, 7(a), and keep at this temp. 1–2 hours. Let stand overnight, filter, and wash free from acid with cold  $\text{H}_2\text{O}$ . Transfer filter to beaker and dissolve in standard NaOH or KOH (1 ml = 0.5 mg of  $\text{P}_2\text{O}_5$ ). Titrate excess of alkali with standard  $\text{HNO}_3$ , using phenolphthalein indicator. Or, after addition of 15 ml molybdate soln, allow to stand 3 hours at temp. not above  $45^\circ$ , filter on small filter or on Gooch crucible, and wash with 2.5%  $\text{NH}_4\text{NO}_3$  soln and then with cold  $\text{H}_2\text{O}$  until two fillings of filter do not diminish greatly color produced with phenolphthalein by 1 drop of standard alkali. Return filter and precipitate to beaker used in the precipitation of phosphomolybdate, dissolve yellow precipitate in standard NaOH or KOH (1 ml = 0.5 mg of  $\text{P}_2\text{O}_5$ ), add few drops of phenolphthalein indicator, II, 10(d), and titrate excess alkali with standard  $\text{HNO}_3$ . Report as percentage of  $\text{P}_2\text{O}_5$ .

23

*Magnesium Nitrate Method*

Place 5 g of prepared soil, 2(a), in porcelain dish. Moisten with 5–7 ml of  $\text{Mg}(\text{NO}_3)_2$  soln, II, 7(e). Dry on water bath and carefully burn organic matter. Cool, and add 10 ml of  $\text{H}_2\text{O}$ , 10 ml of HCl, and 5 ml of  $\text{HNO}_3$ . Cover dish and digest contents 2 hours on water bath, stirring 2 or 3 times during digestion. Dilute to

250 ml, mix well, and filter thru dry folded filter, pouring back thru filter until filtrate is clear. Place aliquot corresponding to 2 or 4 g of soil, depending upon quantity of P present, in hard glass beaker or porcelain dish and evaporate to dryness on water bath. Take up with  $\text{HNO}_3$  (1+4), again evaporate to dryness, and heat 1 hour at 110–120°. Again take up with the dilute  $\text{HNO}_3$  and filter. Reduce combined volume of filtrates and washings to 30–40 ml. Make alkaline with  $\text{NH}_4\text{OH}$  (1+1), and dissolve precipitate with a slight excess of the dilute  $\text{HNO}_3$ . Add gradually with vigorous agitation 15 ml of molybdate soln, II, 7(a). Keep soln at 45° for an hour and then let stand overnight at room temp. Filter, and wash well with 2.5%  $\text{NH}_4\text{NO}_3$  soln and then with cold  $\text{H}_2\text{O}$ . Return filter and precipitate to precipitation flask or beaker and determine P volumetrically as directed under 22. Report as percentage of  $\text{P}_2\text{O}_5$ .

## 24

POTASSIUM AND SODIUM,<sup>10</sup> OR POTASSIUM ONLY

Triturate gently 0.5 or 1 g of impalpably ground soil, 2(b), with 1 g of dry  $\text{NH}_4\text{Cl}$  in an agate mortar, add 8 parts of  $\text{CaCO}_3$ , and mix intimately. Transfer mixture to Pt crucible, rinsing mortar with a little  $\text{CaCO}_3$ . Heat crucible gradually until fumes of  $\text{NH}_4$  salts no longer appear and lower  $\frac{3}{4}$  of crucible is brought to red heat. Maintain this temp. 40–60 min. (Temp. should be sufficient to keep the  $\text{CaCl}_2$ , formed by reaction of  $\text{NH}_4\text{Cl}$  with  $\text{CaCO}_3$ , in state of fusion. The mass does not become a melt because the fused  $\text{CaCl}_2$  is absorbed by the large quantity of  $\text{CaCO}_3$  present. If the silicate is fused by the application of excessive heat, disintegration of mass at end of operation with  $\text{H}_2\text{O}$  cannot be effected. Moreover, excessive temp. induces volatilization of alkali chlorides. The mass contracts in volume during ignition and usually is detached easily from crucible.) Transfer fused mass to porcelain dish, slake with hot  $\text{H}_2\text{O}$ , and grind thoroly with agate pestle. After washing 5 times by decantation with hot  $\text{H}_2\text{O}$ , transfer to filter and wash well (300 ml of wash water is sufficient). To the filtrate add sufficient  $(\text{NH}_4)_2\text{CO}_3$  soln to precipitate any Ca present. Allow to settle, decant supernatant liquid into porcelain dish, and concentrate by evaporation, finally transferring precipitate to dish. When volume is reduced to ca 30 ml, add a little  $(\text{NH}_4)_2\text{CO}_3$  and  $\text{NH}_4\text{OH}$ , heat, filter into porcelain dish, evaporate filtrate to dryness on water bath, and expel  $\text{NH}_4$  salts by ignition; or evaporate with 10 ml of  $\text{HNO}_3$ , followed by two 10 ml additions of  $\text{HCl}$  and evaporations.

If K alone is to be determined, proceed as directed under II, 42(a), beginning, "Dissolve residue in hot  $\text{H}_2\text{O}$ ." Report as percentage of  $\text{K}_2\text{O}$ .

If both K and Na are to be determined, dissolve residual alkali chlorides in 3–5 ml of  $\text{H}_2\text{O}$  (some black or brown flocculent matter usually remains undissolved), warm, and filter thru small filter into weighed Pt dish. Evaporate to dryness on water bath, carefully heat residual alkali chlorides to incipient fusion, cool, and weigh as Na and K chlorides. Dissolve combined chlorides in 30 ml of  $\text{H}_2\text{O}$ , add 1.5 ml of  $\text{PtCl}_4$  soln, II, 40(b), evaporate to sirupy consistency, and add 15 ml of 2.25 N acidulated alcohol (pass  $\text{HCl}$  gas into a mixture of 2000 ml of 95% alcohol and 152 ml of  $\text{HCl}$ ). Filter thru asbestos Gooch and wash with the acidulated alcohol and then with 80% alcohol. Dry Gooch 1 hour in electric oven and weigh. Dissolve  $\text{K}_2\text{PtCl}_6$  with hot  $\text{H}_2\text{O}$ , wash with 80% alcohol, and again place in drying oven for a hour. Cool in desiccator, weigh, and calculate to  $\text{K}_2\text{O}$ . Calculate to  $\text{KCl}$  and deduct from combined weight of Na and K chlorides to obtain  $\text{NaCl}$ . Report as percentage of  $\text{Na}_2\text{O}$ .

## 25

ARSENIC<sup>11</sup>

Weigh 5 g of air-dried soil and transfer to 200 ml Kjeldahl flask. Add 20 ml of  $\text{H}_2\text{SO}_4$  (arsenic free) and thoroly mix acid with soil by rotating flask. Add 5 ml of

$\text{HNO}_3$  (arsenic free) and 0.1 g of  $\text{KClO}_3$  to  $\text{H}_2\text{SO}_4$  soil mixture in flask. Heat flask gently at first, then gradually increase heat until the soln boils, and digest at this temp. until all organic matter is oxidized and acid soln is clear. (For soils of high organic matter content, it may be necessary to add other portions of  $\text{HNO}_3$  to oxidize C and obtain clear soln.) Cool flask. Dilute soln with 50 ml of  $\text{H}_2\text{O}$  and concentrate by boiling until  $\text{SO}_3$  fumes are given off; repeat operation twice to expel all traces of oxides of N. Dilute soln in flask with  $\text{H}_2\text{O}$ , transfer to 100 ml volumetric flask, cool, and make to mark. Take an aliquot and determine arsenic by modified Gutzeit method, XXIX, 1.

SELENIUM<sup>12</sup>

26

## I. For soils

Pulverize air-dried sample with wooden roller until all portions other than rock fragments are disintegrated. Separate rock fragments by use of 2 mm sieve. Sub-

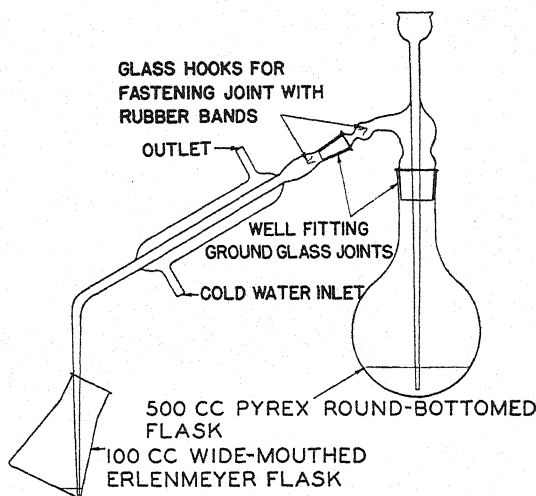


FIG. 4.—APPARATUS FOR DETERMINATION OF SELENIUM IN SOILS

sample sieved material to secure representative sample of 50 g. Transfer weighed material to distilling flask equipped with short condenser and thistle safety tube, all glass connections (Fig. 4). Add 100 ml of  $\text{HBr}$  to which has been added 2 ml of  $\text{Br}$ . Warm gently 15 min. and distil into 100 ml Erlenmeyer flask containing 5 ml of  $\text{H}_2\text{O}$ . Have outlet of distilling tube submerged. If, on gentle warming, a drop of  $\text{Br}$  does not collect beneath  $\text{H}_2\text{O}$  in receiver, add 2 ml of  $\text{Br}$  to distilling flask thru thistle tube and repeat gentle warming. Distil 60 ml into receiver. To distillate in Erlenmeyer add 25 ml of  $\text{H}_2\text{O}$  and cool in iced  $\text{H}_2\text{O}$ . Pass slow stream of  $\text{SO}_2$  into distillate until the  $\text{Br}$  is removed. Add 0.25 g of  $\text{NH}_2\text{OH} \cdot \text{HCl}$ . Warm Erlenmeyer on steam bath at  $80^\circ$  15 min. and allow to stand overnight. The  $\text{Se}$  will appear at bottom of Erlenmeyer as rose-pink precipitate. Modify further treatment according to quantity of precipitate, as directed below.

(a) *Precipitate not greater than 0.5 mg.*—Filter  $\text{Se}$  precipitate thru small asbestos Gooch crucible with suction. If small quantity of oily material accompanies precipitated  $\text{Se}$ , wash asbestos pad with 10 ml of alcohol and then with 10 ml of  $\text{H}_2\text{O}$ .

Redissolve precipitated Se from asbestos pad with 10 ml of 48% HBr, which has been rendered bright red by addition of Br. Collect filtrate by suction in 25 ml volumetric flask and wash pad with 2 portions of H<sub>2</sub>O. Decolorize filtered soln with SO<sub>2</sub> and add 1 ml of a soln containing 100 mg of NH<sub>2</sub>OH.HCl and 25 mg of gum arabic per ml. Make to volume with H<sub>2</sub>O. Transfer flask and contents to steam bath and warm at 80° for 30 min.; cool to room temp., shake vigorously, and transfer to a 50 ml Nessler tube. Before final precipitation of the Se in volumetric flask prepare series of standards in 25 ml volumetric flasks by addition of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 0.7 mg of Se as Na<sub>2</sub>SeO<sub>4</sub>. Precipitate these standards after addition of HBr, Br, H<sub>2</sub>O, NH<sub>2</sub>OH.HCl, and gum arabic, and treat precisely as sample is treated. Compare sample with standard in any suitable color comparator and determine quantity of Se. Express results as p.p.m. of air-dried soil.

(b) *Precipitate below 1 p.p.m. (established by preceding determination) and for greater precision.*—Distil 100 g of sample with HBr, 26. When distillation is complete, replace sample in distillation flask with a second 100 g sample. Add to distillate from first distillation, 50 ml additional HBr and 2–4 ml of Br, together with 22 ml of H<sub>2</sub>SO<sub>4</sub>. Allow to stand and repeat distillation as often as necessary to integrate the minute quantities of Se until an adequate quantity for measurement is obtained.

(c) *Initial precipitate in excess of 0.5 mg.*—Redissolve washed precipitate in HBr, colored with Br as directed under (a). Transfer dissolved material to a 100 ml beaker and dilute with 20% HBr to volume of 50 ml. Precipitate this soln with SO<sub>2</sub> and add 0.25 g of NH<sub>2</sub>OH.HCl. Warm on steam bath 15 min. and allow to stand overnight at room temp. Filter on weighed Gooch, dry 4 hours at 85°, and weigh. (Balance used must be sensitive to at least 0.05 mg.)

27

## II. For shales and sulfide ores

Place 200 ml of HNO<sub>3</sub> in 400 ml porcelain evaporating dish and heat to gentle boiling. Add slowly 10 g of the powdered sample. If ensuing reaction is vigorous, add sample in small quantities and allow reaction to subside after each addition. After entire sample has been added, add 50 ml of H<sub>2</sub>SO<sub>4</sub> and allow mixture to stand on water bath overnight. Transfer to distillation flask and proceed as directed in 26, 26(a), or 26(b). If shales are free from sulfides, treat as prescribed in 26.

## TOTAL BORON<sup>2</sup>

28

### REAGENTS

(a) *Methyl alcohol.*—Reflux synthetic methanol overnight with lime and distil.

(b) *Phosphoric acid.*—C. P. 85% and B-free.

(c) *Anhydrous sodium dihydrogen phosphate.*—Dry the hydrated salt at 110° and pulverize.

(d) *Bromothymol blue indicator.*—Dissolve 1 g of bromothymol blue in 100 ml of H<sub>2</sub>O and add 0.5 N NaOH until indicator just dissolves and soln is neutral.

(e) *Standard boric acid soln.*—Dissolve 1.429 g of C. P. boric acid in 1000 ml of H<sub>2</sub>O. 1 ml = 0.25 mg of B.

(f) *Standard sodium hydroxide soln.*—Prepare ca 0.02 N soln from concentrated carbonate-free NaOH soln. Standardize with boric acid soln.

(g) *Mannite.*—Neutral C. P. mannite (mannitol).

Use only B-free glassware.

29

### DETERMINATION

Mix 10–25 g of soil, ground to pass 100-mesh sieve, with 3–4 times (depending on composition of soil) this quantity of the Na dihydrogen phosphate in 200 ml iron

crucible. Slowly fuse mixture over open flame generated by three Meker burners, rotating molten mass frequently to insure intimate contact between soil and flux. If fused material is too viscous, cool, add 5–10 ml of phosphoric acid, and refuse. Cool, detach melt from crucible, pulverize, dust into 100 ml of the 85% phosphoric acid in a 500 ml round-bottomed flask, with frequent shaking, and heat on steam bath overnight. If 20 g or more soil is used, divide pulverized fused mass into two portions and decompose and distil separately, combining distillates.

Add 50 ml of methyl alcohol and connect flask with condenser, using a distilling head, and with inlet tube from water-jacketed flask containing methyl alcohol. Distil as in steam distillation, and collect ca 500 ml of alcohol in receiver containing 5–10 ml of 2 *N* NaOH. Distil alcohol from alkaline distillate, transfer residue to Pt dish, evaporate to dryness, and ignite gently. Dissolve residue in H<sub>2</sub>O and 2 *N* HCl, transfer to flask or beaker, make slightly acid to bromothymol blue with HCl, and boil to expel CO<sub>2</sub>. Cool soln, adjust to faint blue tinge of indicator with the standard NaOH and 0.1 *N* HCl and read buret. Add 5 g of mannite and titrate with standard alkali back to same end point. For highest accuracy titrate electrometrically; calomel and glass electrodes are suitable. Adjust soln to pH of ca 7 for initial and final end points.

30

## ACID-SOLUBLE BORON

Digest 50 g of soil, passing 2-mm sieve, overnight on steam bath with 75–100 ml (depending on composition of soil) of 85% phosphoric acid in 500 ml flask. Proceed as directed under 29, second paragraph.

FLUORINE<sup>14</sup>

31

## REAGENTS

- (a) *Calcium peroxide*.—Of known F content.
- (b) *Phenolphthalein*.—0.1% in 1+1 alcohol.
- (c) *Sodium alizarin sulfonate*.—0.05% aqueous soln.

32

## DETERMINATION

For soils of high F content, use 0.5 g charge; for those low in F, use 1 g. Mix charge intimately with 3 times its weight of the CaO<sub>2</sub> in either Ni or Pt crucible. Char thoroly below 500°, and then heat at 900° for 30 min. Cool, and transfer the incinerate into distillation flask devoid of silica coating. Wash walls of flask with 5 ml. of H<sub>2</sub>O; add 3 drops of the phenolphthalein; neutralize with 60% perchloric acid, and then add 15 ml more. Connect distillation flask with condenser and bring suspension to 135, ±5°; maintain distillation temp. and volume during collection of 200–250 ml of distillate, while passing current of steam thru suspension. Make distillate to 250 ml.

Use 25 ml aliquot, diluted with equal volume of alcohol. Introduce 10 drops of the sodium alizarin sulfonate; neutralize with 0.05 *N* NaOH and then adjust pH to 3.0, ±0.2, with 2.5 ml of 0.05 *N* HCl. Titrate with 0.01 *N* thorium nitrate to faint pink end point corresponding to that of blank. Express results as p.p.m. of F.

## REPLACEABLE BASES IN SOILS DEVOID OF CARBONATES

33

## REAGENTS

*Neutral normal ammonium acetate*.—Prepare approximately 2 *N* NH<sub>4</sub>OH from C. P. concentrated NH<sub>4</sub>OH and 2 *N* acetic acid from glacial acetic acid. Mix the two



solns and adjust to pH 7.0 by bromothymol blue or by glass electrode. Keep in stoppered bottle.

34

## DETERMINATION

Weigh 10 g of 0.5 mm air-dry soil into 250 or 300 ml Pyrex Erlenmeyer flask; introduce 100 ml of the  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln, stopper, and shake vigorously 2–3 seconds. Let stand 1 hour or longer, agitating every 15 min. Filter on 70 mm Büchner filter with light suction. Transfer soil from flask to filter and leach with the  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  to total volume of 250 ml. Transfer filtrate to 400 ml Pyrex beaker and save residue for determination of exchange capacity. Evaporate filtrate and bake to dryness on hot plate. Ignite organic matter with Bunsen burner, heating sides and bottom of beaker to avoid spattering.

Dissolve residue with excess of 0.1 N HCl and add 2 drops of methyl orange (0.1% aqueous soln), avoiding undue excess of the HCl by letting the acid digest slowly on hot plate in covered beaker; add more HCl if necessary, as indicated by change in the methyl orange. (Dissolution is complete when liquid appears free of solids other than fine particles of C.) Filter insoluble matter, wash beaker and filter 5–6 times. Back titrate with 0.1 N NaOH and record net acid in terms of 0.1 N. On basis of 10 g charge, each ml net acid used is equal to 1 milliequivalent replaceable bases per 100 g of soil.

35

*Replaceable Calcium*

To soln from 34 add 10 ml of HCl, make ammoniacal with  $\text{NH}_4\text{OH}$  (1+1), and determine Ca as directed under 14(c). Each ml of 0.1 N  $\text{KMnO}_4$  = 1 milliequivalent of Ca per 100 g of soil.

*Replaceable Potassium*<sup>15</sup>

36

## REAGENTS

(a) *Sodium cobaltinitrite*.—Dissolve 30 g of  $\text{Na}_3\text{CO}(\text{NO}_2)_6$  in 100 ml of  $\text{H}_2\text{O}$ , and filter thru asbestos on Gooch crucible. Keep in glass-stoppered bottle at 10°.

(b) *Acetic acid*.—Approximately 0.15 N. Dilute 9 ml of 99% acetic acid to 1000 ml with  $\text{H}_2\text{O}$ .

(c) *Asbestos*.—Digest ca 24 hours in  $\text{HNO}_3$  (1+9) with enough permanganate to maintain deep purple color.

(d) *Sodium hydroxide*.—10 g of C. P. NaOH in 90 ml of  $\text{H}_2\text{O}$ .

37

## DETERMINATION

Evaporate filtrate from 35 on hot plate until crust begins to form. Add 15 ml of  $\text{HNO}_3$ , cover beaker, and evaporate to dryness on hot plate. Wash cover-glass and sides of beaker and again evaporate to dryness. Wash down all traces of  $\text{NH}_4$  condensation, add 1–2 drops of the NaOH, and evaporate to dryness. Cool, and add 20 ml of acetic acid. Police bottom and sides of beaker; filter, and wash 3 times with 5 ml of  $\text{H}_2\text{O}$ . Cool to at least 10° and add, with stirring, 5 ml of the Na cobaltinitrite. Allow to stand overnight at temp. below 10° and then wash precipitate 5 times. Remove crucible and wipe outside free of any adhering reagent. Place crucible in original beaker and just cover with hot  $\text{H}_2\text{O}$ . Add few drops of 0.05 N  $\text{KMnO}_4$  from buret and then introduce 5 ml of  $\text{H}_2\text{SO}_4$  (1+4). Titrate with the  $\text{KMnO}_4$ , timing stirring with reduction of the  $\text{KMnO}_4$  so as to maintain a slight excess during this step. Remove crucible, rinse with hot  $\text{H}_2\text{O}$ , place beaker over low flame, and digest near boiling 2–3 min. Assure that permanganate continues in slight excess. Remove beaker from flame, add enough oxalic acid to discharge purple color, and complete

titration with the permanganate. Based on 10 g charge, 1 ml of 0.05  $N$   $KMnO_4$  = 0.0003259 g of K, or 0.08 milliequivalents per 100 g of soil.

38

*Replaceable Magnesium*

(Applicable only to soils of meager sodium content characteristic of humid regions.)

*I. Determination by difference.*—Subtract the sum of Ca, 35, and K, 36, milliequivalent from total milliequivalents of replaceable bases. Difference = replaceable Mg.

*II. Direct determination.*—Evaporate filtrate from 35 to crust formation on hot plate. Add 15 ml of  $HNO_3$ , cover beaker, and evaporate to dryness. Add 10 ml of  $HCl$  (1+1), warm 5 min. on hot plate, and wash cover and sides of beaker. Make slightly ammoniacal, heat to boiling, add 0.2–0.3 g of  $(NH_4)_2S_2O_8$ , and digest on hot plate to flocculate Mn, maintaining alkalinity by frequent dropwise additions of  $NH_4OH$  (1+1) to maintain slight ammoniacal odor. Filter on 7 cm filter, rinse beaker, and wash filter 3–4 times with hot slightly ammoniacal  $NH_4Cl$  (2%). To filtrate add 2 ml of a 10% soln of  $(NH_4)_2HPO_4$  and also add sufficient  $NH_4OH$  to make alkaline to methyl red. Allow to stand 20 min., add  $NH_4OH$  ( $\frac{1}{8}$  volume), stir until precipitate appears, and allow to stand overnight. Filter on 7 cm paper; rinse beaker 3 times and wash filter 4 times with dilute alcohol (1+1). Remove filter, open, and place against side of beaker; wash precipitate from whole filter into aqueous suspension (50 ml); add 1 drop of 1% bromocresol green, and deliver from buret, with stirring, enough 0.1  $N$   $H_2SO_4$  to impart a permanent yellow color. Titrate with 0.1  $N$   $NaOH$  to first shade of blue. Each ml of net acid = 0.00202 g of  $MgO$ , or 1 milliequivalent Mg per 100 g of soil, basis of 10 g charge.

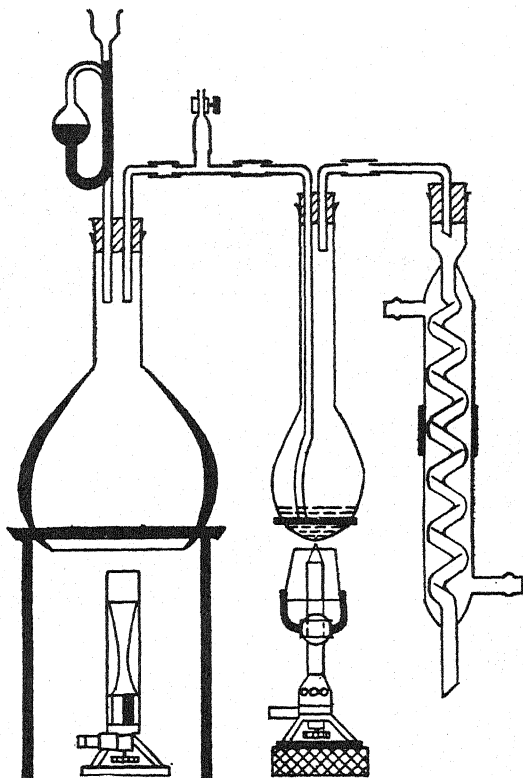


FIG. 5.—STEAM DISTILLATION APPARATUS

## AVAILABLE BASES IN CALCAREOUS SOILS

*Extraction with Boiling Ammonium Chloride*

39

## REAGENT

*Ammonium chloride.*—2  $N$   $NH_4Cl$ , from C. P. salt.

## 40

## APPARATUS

Steam distillation apparatus, Fig. 5, consisting of 3 liter insulated Pyrex boiling flask, 500 ml Pyrex long-necked Kjeldahl, 14" coiled condenser, and glass connections of 7 mm glass tubing. T-tube connecting the flasks is for release of steam pressure. Tip of condenser should be provided with a piece of rubber tubing reaching to bottom of receiver.

## 41

## DETERMINATION

Weigh 10 g of 0.5 mm air-dried soil into Kjeldahl flask; introduce 100 ml of the  $\text{NH}_4\text{Cl}$ , connect to steam digestion apparatus (Fig. 5), and continue distillation until 100 ml distillate titrates only 1.2 ml of 0.1  $N$   $\text{NH}_4\text{OH}$ . Maintain heat under digestion flask so that volume shall not exceed 100 ml. Remove flask and rinse connecting tubes into flask; cool, add 100 ml of  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ , 33, and 5 drops of  $\text{NH}_4\text{OH}$  (1+1) and shake gently. Filter on 70 ml Büchner filter. Use the  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  to transfer soil onto filter, and wash residue with additional 100 ml. Transfer filtrate to 400 ml Pyrex beakers and save residue for exchange capacity determination. Evaporate filtrate on hot plate until crust appears to form on side of beaker. Remove from hot plate, add 25 ml of  $\text{HNO}_3$ , place cover-glass over beaker, and continue evaporation to dryness. Wash sides of beaker and dissolve residue by warming on hot plate. Add, while swirling, 5 ml of 10% oxalic acid. Evaporate to dryness on steam bath or below  $100^\circ$  on hot plate. If inside of beaker is not frosted from oxalic acid condensation, add 5 ml more oxalic acid and again evaporate. Wash sides of beaker and again evaporate to dryness. Ignite in muffle furnace at  $550^\circ$  or over Bunsen burner, taking care that heat has been applied to all spots where precipitate occurred.

Dissolve residue as directed in 34, beginning "Dissolve residue," and designate this as "total available bases."

## 42

*Available Calcium.*—See 35.

## 43

*Replaceable Potassium.*—See 37.

## 44

*Available Magnesium.*—See 38.

## 45

*Replaceable Calcium*

Determine by difference, as follows:

(a) If total available Mg is comparatively low, subtract the carbonate equivalence, 6 or 7, from available Ca, 42, and designate difference as replaceable Ca.

(b) If available Mg approximates Ca, subtract one-half of carbonate equivalence from total available Ca, 42, and designate difference as replaceable Ca.

## 46

*Replaceable Magnesium*

(a) If condition 45(a) obtains, replaceable Mg = available Mg.

(b) If condition 45(b) obtains, subtract one-half of carbonate equivalence, 45(b) and designate difference as replaceable Mg.

*Exchange Capacity*

## 47

## REAGENTS

(a) *Ethanol.*—U.S.P. 95%. Should react alkaline to phenolphthalein upon addition of 0.1 ml of 0.1  $N$   $\text{NaOH}$  per 100 ml.

(b) *Hydrochloric acid.*—0.1  $N$ . Delivered from automatic zero-point buret fitted into acid reservoir.

(c) *Methyl orange*.—0.1 % aqueous soln.

(d) *Anti-foam*.—Mixture of mineral oil and caprylic alcohol.

48

## APPARATUS

Same as 40, except that stopper fitted to Kjeldahl flask is provided with a small separatory funnel for introduction of alkali. Wash the Kjeldahl flask and its connections free of  $\text{NH}_4$  salts immediately before use.

49

## DETERMINATION

Wash soil from 34 or 41 with ethanol in portions of 15 ml to total of 250 ml. Drain free of alcohol and transfer immediately to distillation flask. Add few drops of the anti-foam and connect flask with steam distillation apparatus (Fig. 5). Place receiver containing 10 ml of the HCl and 2 drops of the methyl orange under condenser. Dip the condenser outlet into the acid in receiver, start steam passage thru soil suspensions and continue until all air is displaced from distillation flask. Introduce 12–15 ml of approximately normal NaOH into the separatory funnel. Relieve steam pressure by opening at T, and deliver the alkali slowly from the separatory funnel into distillation flask without admitting air. Close steam line and continue distillation. Stir acid in receiver frequently during the first minute or two to assure that ammoniacal liquor does not rise to surface. Add additional quantity of the HCl if indicator shows insufficiency. Continue distillation until 200 ml of distillate is collected. Complete titration by first adding enough of the NaOH to make the distillate distinctly alkaline, and finish titration to first change from clear yellow. Ml of acid used per 10 g charge = milliequivalents of absorbed  $\text{NH}_4$  per 100 g soil, or *exchange capacity*.

50

*Replaceable Hydrogen*

Obtain by subtracting replaceable bases, 34, or the sum of 35, 37, and 38, from absorbed  $\text{NH}_4$ , 49.

## HYDROGEN-ION CONCENTRATION OF SOILS

*I. Of Humid Regions*

51

## APPARATUS

Use an accepted standard potentiometer provided with a glass electrode.

52

## DETERMINATION

To ca 50 g of fresh soil, add 50 ml of  $\text{H}_2\text{O}$  and let stand 30 min. with intermittent agitation. Determine pH value on part, or whole, of this suspension by the electro-metric method with a glass electrode.

53

*II. Of Arid and Semi-arid Regions*

Weigh 20–25 g of soil into 50 ml beaker and add boiled  $\text{H}_2\text{O}$  carefully until soil is soft enough to admit ready penetration of electrodes. (Resultant moisture content is slightly above the moisture equivalent and well below the water-holding capacity of the soil. The mass may be stirred with glass rod to produce a uniform mass.) Tap beaker gently on table top, press glass electrode and its companion calomel electrode into soil, and make the pH reading. Make several readings on each sample, withdrawing electrodes and pressing them again into soil mass. Initial reading is

often inaccurate, since complete equilibrium is not always attained by the first contact between the electrodes and the soil mass.

## 54

## QUALITATIVE TEST FOR SOIL REACTION

Place strips of neutral litmus paper in bottom of a number of Petri dishes; over these lay 1 or 2 thicknesses of filter paper (free from acid); and place prepared soil, 2(a), on filter paper. With horn spoon or clean spatula press soil down firmly against paper and add enough  $H_2O$  (tested and found neutral) to saturate soil. Cover dishes, allow to stand 30 min., and note color of test paper. Have a check Petri dish containing neutral litmus paper and filter paper, moistened with the same  $H_2O$ , stand under same conditions. (Filter paper gives a uniform background and evenness of contact.)

## REACTION VALUES

## 55

DETERMINATION<sup>16</sup>

Use either colorimetric or electrometric method, as found convenient. Determine reaction values on fresh moist samples, using soil-to-water ratio of 1:5, with intermittent agitation for 30 min.

## 56

## METHOD OF STATEMENT

For simplicity and ease of interpretation use a dual system of statement, giving both pH values and their equivalents in arithmetically related numbers in parentheses, as tabulated.

SØRENSEN OR VALUES H	SPECIFIC ACIDITY	SØRENSEN OR pH VALUES	SPECIFIC ALKALINITY
7.0	0.0	7.0	0.0
6.9	0.5	7.1	0.5
6.8	1.0	7.2	1.0
6.7	1.5	7.3	1.5
6.6	2	7.4	2
6.5	3	7.5	3
6.4	4	7.6	4
6.3	5	7.7	5
6.2	6	7.8	6
6.1	8	7.9	8
6.0	10	8.0	10
5.9	12.5	8.1	12.5
5.8	16	8.2	16
5.7	20	8.3	20
5.6	25	8.4	25
5.5	31.5	8.5	31.5
5.4	40	8.6	40
5.3	50	8.7	50
5.2	63	8.8	63
5.1	80	8.9	80
5.0	100	9.0	100
4.9	125	9.1	125
4.8	160	9.2	160
4.7	200	9.3	200
4.6	250	9.4	250
4.5	315	9.5	315

## 57

## NITRATE NITROGEN

Place 100 g of prepared air-dried soil, 2(a), and 500 ml of  $H_2O$  in suitable container, and agitate 5 min. Add 1 g of  $CaO$  or 2 g of precipitated  $CaCO_3$ , agitate thoroly, allow to stand 10–20 min., and obtain a clear filtrate. If filtrate contains

6 p.p.m. of Cl, or less, proceed as directed under XXXVII, 17, using 25 ml of filtrate; if it contains more than 6 p.p.m. of Cl, proceed as directed under XXXVII, 19, using 25 ml or volume containing N not in excess of 0.1 mg of N in form of nitrate. Report as percentage of nitrate N.

58

## ALKALI SALTS

To 100 g of soil in 500 ml bottle, add 250 ml of  $H_2O$ . Stopper, shake thoroly, and allow to stand overnight. Filter thru Pasteur-Chamberland filter. Evaporate 50 ml of filtrate to dryness in Pt dish on steam bath, ignite gently to decompose organic matter, cool in desiccator, and weigh for total salts. Dissolve residue in the Pt dish in 10–15 ml of hot  $H_2O$ , transfer to 50 ml volumetric flask, cool, and dilute to mark.

For determination of Cl, titrate an aliquot of 10 ml against 0.1 N  $AgNO_3$  soln. Report as percentage of NaCl.

For determination of alkali carbonate, titrate aliquot of 10 ml against 0.1 N HCl. Report as percentage of  $Na_2CO_3$ .

Determine sulfates by difference. If much  $CaSO_4$  is present, filter 10 ml aliquot of the soln of the salts in hot  $H_2O$  thru a small filter, add 75 ml of alcohol, and digest 3 hours; filter, wash with alcohol, and ignite; subtract amount of  $CaSO_4$  from amount of sulfates.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 4, 289 (1920).
- <sup>2</sup> J. Ind. Eng. Chem., 7, 227 (1915).
- <sup>3</sup> J. Assoc. Official Agr. Chem., 14, 283 (1931).
- <sup>4</sup> J. Ind. Eng. Chem., 8, 637 (1916).
- <sup>5</sup> U. S. Geol. Survey Bull., 700, p. 94.
- <sup>6</sup> J. Ind. Eng. Chem., 9, 1114 (1917).
- <sup>7</sup> Washington, Chemical Analysis of Rocks, 3rd ed., 1919, p. 181.
- <sup>8</sup> J. Assoc. Official Agr. Chem., 14, 138 (1931); 16, 43 (1933); 19, 66 (1936); Ind. Eng. Chem., Anal. Ed., 4, 214 (1932).
- <sup>9</sup> Ind. Eng. Chem., 15, 1183 (1923).
- <sup>10</sup> Fresenius, Quantitative Chemical Analysis, Revised and amplified translation of the 6th German ed., 1906, vol. 2, p. 1175; Crookes, Select Methods in Chemical Analysis, 4th ed., 1905, p. 23; Wiley, Principles and Practice of Agricultural Analysis, 1906, Vol. 1, 423; U. S. Geol. Survey Bull., 700, p. 207.
- <sup>11</sup> J. Assoc. Official Agr. Chem., 18, 62, 207 (1935).
- <sup>12</sup> Ibid., 19, 312 (1936).
- <sup>13</sup> Am. Fertilizer, Nov. 25, 1939.
- <sup>14</sup> J. Assoc. Official Agr. Chem., 16, 105 (1933); 19, 156 (1936); 22, 231 (1939).
- <sup>15</sup> J. Am. Soc. Agron., 26, 537 (1934).
- <sup>16</sup> J. Wash. Acad. Sci., 9, 305 (1919); 11, 197 (1921); Ecology, 3, 346 (1922); Am. J. Pharm., 2, 59 (1927); J. Assoc. Official Agr. Chem., 12, 64 (1929).

## II. FERTILIZERS

1

### DIRECTIONS FOR SAMPLING<sup>1</sup>—OFFICIAL

Each official sample shall consist of at least 1 lb. of material taken in following manner: Use sampler that removes a core from top to bottom of bag. Take cores from not less than 10% of bags present unless this process necessitates cores from more than 20 bags, in which case take core from 1 bag for each additional ton represented. If less than 100 bags, sample not less than 10 bags; if less than 10 bags, sample all bags. Thoroughly mix portions taken on clean oilcloth or paper, reduce by quartering to quantity of sample required, and place in air-tight container.

2

### PREPARATION OF SAMPLE—OFFICIAL

Pass entire sample thru 10-mesh sieve before subdividing for analysis. Reduce gross sample by quartering to quantity sufficient for analytical purposes. Transfer sample to sieve having circular openings 1/25" (1 mm) in diameter and sift, breaking the lumps with a pestle. Grind portion remaining on sieve until all particles pass thru, grinding and sifting as rapidly as possible to avoid loss or gain of moisture during operation. Mix thoroughly and preserve in tightly stoppered bottles.

3

### MECHANICAL ANALYSIS OF BONE AND TANKAGE—OFFICIAL

Transfer 100 g of original material to sieve having circular openings 0.5 mm in diameter. Sift, breaking lumps by means of soft rubber pestle if material has tendency to cake. Weigh coarse portion remaining on sieve. Determine fine portion by difference.

### MOISTURE

4

#### *By Drying—Official*

Heat 2 g of prepared sample, 2, for 5 hours in water oven at temp. of boiling H<sub>2</sub>O (98–100°). In case of potash salts, NaNO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, heat at ca 130° to constant weight. Report percentage loss in weight as moisture.

#### *By Distillation with Toluene<sup>2</sup>—Tentative\**

5

### APPARATUS

(a) *Distillation apparatus.*—250 ml Pyrex Erlenmeyer flask, receiving tube or trap graduated in 0.1 ml, and condenser. Ground-glass joints are preferable, but pressed cork may be used, XXVII, 3.

(b) *Tube brush attached to a long wire.*

6

### DETERMINATION

Place in flask sufficient sample to give 2–5 ml of H<sub>2</sub>O (weight should not exceed 20 g). Weigh rapidly to 1 mg. (Extremely hygroscopic materials should be placed in covered weighing tubes, and samples should be weighed out by difference.) If sample is likely to bump, add enough dry sand to cover bottom of flask. Add 100 ml of toluene immediately and connect flask to trap and condenser. Pour 50 ml of toluene thru condenser, filling trap. Bring mixture to boil and distil slowly (ca 2

\* Adopted as applicable to urea, calcium nitrate, ammonium nitrate, and other salts containing water of crystallization, or that are volatile or decomposed at low temperature, and to mixtures containing such salts. (*Jour. A.O.A.C.*, 15, 66 (1932).) Recommended for further study of applicability (*Ibid.*, 23, 51 (1940)).

drops per second), until most of  $\text{H}_2\text{O}$  has passed over, then increase rate of distillation to ca 4 drops per second. When all  $\text{H}_2\text{O}$  has apparently distilled, wash down condenser by pouring 5–10 ml of toluene in at top, and continue distillation until no more  $\text{H}_2\text{O}$  will distil over. (Distillation for organic materials and salts that contain no water of crystallization may be completed within an hour, but such salts as calcium nitrate require much longer time, 7–10 hours.) If  $\text{H}_2\text{O}$  remains in condenser, remove by brushing down with tube brush attached to long wire, washing down condenser at same time with toluene. Allow receiving tube to come to room temp. Force any drops adhering to sides of tube into  $\text{H}_2\text{O}$  column with a rubber band wrapped around a wire. Read volume of  $\text{H}_2\text{O}$  and calculate percentage of sample, assuming weight of 1 ml of  $\text{H}_2\text{O}$  to be 1 g at room temp.

It is necessary to have condenser and receiving tube absolutely clean at start to prevent adherence of  $\text{H}_2\text{O}$  to glass. Clean with chromic-sulfuric acid, rinse with alcohol, and dry thoroly. Used toluene may be recovered by distillation from anhydrous  $\text{CuSO}_4$ .

#### TOTAL PHOSPHORIC ACID

##### *Gravimetric Method—Official*

7

#### REAGENTS

(a) *Molybdate soln.*—Dissolve 100 g of  $\text{MoO}_3$  in a mixture of 144 ml of  $\text{NH}_4\text{OH}$  and 271 ml of  $\text{H}_2\text{O}$ . Cool, and pour soln slowly and with constant stirring into a cool mixture of 489 ml of  $\text{HNO}_3$  and 1148 ml of  $\text{H}_2\text{O}$ . Keep final mixture in a warm place for several days or until portion heated to  $40^\circ$  deposits no yellow precipitate of  $\text{NH}_4$  phosphomolybdate. Decant soln from any sediment and preserve in glass-stoppered vessels.

(b) *Ammonium nitrate soln.*—Dissolve 100 g of phosphate-free  $\text{NH}_4\text{NO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(c) *Magnesia mixture.*—(1) Dissolve 11 g of  $\text{MgO}$  in  $\text{HCl}$  (1+4), avoiding an excess of the acid; add a little  $\text{MgO}$  in excess; boil few minutes to precipitate  $\text{Fe}$ ,  $\text{Al}$ , and  $\text{P}_2\text{O}_5$ ; and filter. To filtrate add 140 g of  $\text{NH}_4\text{Cl}$  and 130.5 ml of  $\text{NH}_4\text{OH}$  and dilute to 1 liter. Or, (2) dissolve 55 g of crystallized  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , add 140 g of  $\text{NH}_4\text{Cl}$  and 130.5 ml of  $\text{NH}_4\text{OH}$ , and dilute to 1 liter. Or, (3) dissolve 55 g of crystallized  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , add 140 g of  $\text{NH}_4\text{Cl}$ , and dilute to 870 ml. Add  $\text{NH}_4\text{OH}$  to each required portion of soln just before using, at rate of 15 ml per 100 ml of soln.

(d) *Ammonium hydroxide for washing* (1+9).—Should contain not less than 2.5% of  $\text{NH}_3$  by weight.

(e) *Magnesium nitrate soln.*—Dissolve 150 g of  $\text{MgO}$  in  $\text{HNO}_3$  (1+1), avoiding an excess of acid; add a little  $\text{MgO}$  in excess, boil, filter from excess of  $\text{MgO}$ ,  $\text{Fe}_2\text{O}_3$ , etc., and dilute to 1 liter.

8

#### PREPARATION OF SOLUTION

Treat 2 g of sample by one of methods given below. Cool soln, dilute to 200 ml, mix, and pour on dry filter.

(a) Dissolve in 30 ml of  $\text{HNO}_3$  and 3–5 ml of  $\text{HCl}$  and boil until organic matter is destroyed. (Suitable for materials containing small quantity of organic matter.)

(b) Dissolve in 15–30 ml of  $\text{HCl}$  and 3–10 ml of  $\text{HNO}_3$ . (Recommended for fertilizers containing much  $\text{Fe}$  or  $\text{Al}$  phosphate.)

(c) Evaporate with 5 ml of  $\text{MgNO}_3$ , ignite, and dissolve in  $\text{HCl}$ . (Suitable for organic material like cottonseed meal alone or in mixtures.)

(d) Boil with 20–30 ml of  $\text{H}_2\text{SO}_4$  in 200 ml flask, adding 2–4 g of  $\text{NaNO}_3$  or  $\text{KNO}_3$ ,



at beginning of digestion and small quantity after soln has become nearly colorless, or adding the nitrate in small portions from time to time. When soln is colorless, add 150 ml of  $H_2O$  and boil few minutes. (Generally applicable to materials or mixtures containing large quantities of organic matter. With cottonseed meals and materials of like nature it is best to add first ca 5 ml of  $HNO_3$  and then the  $H_2SO_4$ .) Before adding the nitrate, allow mixture to digest, at a gentle heat if necessary, until violence of reaction is over.

9

## DETERMINATION

Pipet aliquot of prepared soln corresponding to 0.25 g, 0.50 g, or 1 g, into a 250 ml beaker; add  $NH_4OH$  in slight excess, and barely dissolve precipitate formed with few drops of  $HNO_3$ , stirring vigorously. If  $HCl$  or  $H_2SO_4$  has been used as solvent, add ca 15 g of crystalline  $NH_4NO_3$  or a soln containing that quantity. To hot soln add 70 ml of the molybdate soln for every decigram of  $P_2O_5$  present. Digest at ca  $65^\circ$  for 1 hour, and determine whether or not the  $P_2O_5$  has been completely precipitated by adding more molybdate soln to clear supernatant liquid. Filter, and wash with cold  $H_2O$  or preferably with the  $NH_4NO_3$  soln. Dissolve precipitate on filter with  $NH_4OH$  (1+1) and hot  $H_2O$  and wash into beaker to volume of not more than 100 ml. Neutralize with  $HCl$ , using litmus paper or bromothymol blue as indicator; cool; and from buret add slowly (ca 1 drop per second), stirring vigorously, 15 ml of the magnesia mixture for each decigram of  $P_2O_5$  present. After 15 min. add 12 ml of  $NH_4OH$ . Let stand until supernatant liquid is clear (usually 2 hours), filter, wash precipitate with  $NH_4OH$  (1+9) until the washings are practically free from chlorides, dry, burn at low heat, and ignite to constant weight, preferably in electric furnace, at  $950-1000^\circ$ ; cool in desiccator, and weigh as  $Mg_2P_2O_7$ . Report as percentage of  $P_2O_5$ .

*Volumetric Method—Official*

10

## REAGENTS

(a) *Molybdate soln.*—To 100 ml of molybdate soln, 7(a), add 5 ml of  $HNO_3$ . Filter this soln immediately before using.

(b) *Standard sodium or potassium hydroxide soln.*—Dilute 323.81 ml of  $N$  alkali, free from carbonates, to 1 liter; 100 ml of the soln should neutralize 32.38 ml of  $N$  acid; 1 ml = 1 mg or 1% of  $P_2O_5$  on basis of 0.1 g of substance.

(c) *Standard acid soln.*—Prepare soln of  $HCl$  or of  $HNO_3$ , corresponding to strength of (b), or to  $\frac{1}{2}$  of this strength, and standardize by titration against that soln, using the phenolphthalein indicator.

(d) *Phenolphthalein indicator.*—Dissolve 1 g of phenolphthalein in 100 ml of alcohol, 95% by volume.

11

## PREPARATION OF SOLUTION

Treat 2 g of sample as directed under 8(a), (b), (c) or (d), preferably (a) when these acids are a suitable solvent, and dilute to 200 ml with  $H_2O$ .

12

## DETERMINATION

(a) For percentages up to 5 use an aliquot corresponding to 0.4 g of substance; for percentages between 5 and 20 use an aliquot corresponding to 0.2 g of substance; and for percentages above 20 use an aliquot corresponding to 0.1 g of substance. Add 5–10 ml of  $HNO_3$ , depending on method of soln (or equivalent in  $NH_4NO_3$ );

add  $\text{NH}_4\text{OH}$ \* until precipitate that forms dissolves but slowly on stirring vigorously, dilute to 75–100 ml, and adjust to temp. of 25–30°. For percentages below 5, add 20–25 ml of the freshly filtered molybdate soln; for percentages between 5 and 20, add 30–35 ml of the molybdate soln; and for percentages greater than 20, add sufficient molybdate soln to insure complete precipitation. Place soln in shaking or stirring apparatus and shake or stir 30 min. at room temp., decant *at once* thru filter, and wash precipitate twice by decantation with 25–30 ml portions of  $\text{H}_2\text{O}$ , agitating thoroly and allowing to settle. Transfer precipitate to filter and wash with cold  $\text{H}_2\text{O}$  until filtrate from 2 fillings of filter yields pink color upon addition of phenolphthalein and 1 drop of the standard alkali. Transfer precipitate and filter to the beaker or precipitating vessel, dissolve precipitate in small excess of the standard alkali, add few drops of phenolphthalein indicator, and titrate with the standard acid.

(b) *Not applicable in the presence of sulfates.*<sup>3</sup>—Proceed as directed under (a) to a point where soln is diluted to 75–100 ml. Heat in water bath to 45–50°, add the molybdate soln at rate of 75 ml for each decigram of  $\text{P}_2\text{O}_5$  present, and allow mixture to remain in bath, stirring occasionally, 30 min. Decant *at once* thru filter, wash, and titrate as directed under (a).

#### WATER-SOLUBLE PHOSPHORIC ACID

13

##### *Gravimetric Method—Official*

Place 1 g of sample on 9 cm filter and wash with successive small portions of  $\text{H}_2\text{O}$  until filtrate measures ca 250 ml. Allow each portion of wash  $\text{H}_2\text{O}$  to pass thru filter before adding more and use suction if the washing would not otherwise be complete within 1 hour. If filtrate is turbid, add 1–2 ml of  $\text{HNO}_3$ . Dilute to convenient volume, mix well, and proceed as directed under 9.

14

##### *Volumetric Method—Official*

Treat sample as directed under 13. To aliquot of soln corresponding to 0.1, 0.2, or 0.4 g, add 10 ml of  $\text{HNO}_3$ , nearly neutralize with  $\text{NH}_4\text{OH}$ , dilute to 60 ml, and proceed as directed under 12.

#### CITRATE-INSOLUBLE PHOSPHORIC ACID—OFFICIAL

15

##### REAGENTS

*Ammonium citrate soln.*<sup>4</sup>—Should have a sp. gr. of 1.09 at 20° and pH of 7.0 as determined by electrometric method with hydrogen electrode or by colorimetric method with phenol red. When using colorimetric method proceed as follows:

Dissolve 370 g of crystallized citric acid in 1500 ml of  $\text{H}_2\text{O}$  and nearly neutralize by adding 345 ml of  $\text{NH}_4\text{OH}$  soln (28–29%  $\text{NH}_3$ ). If concentration of the  $\text{NH}_4\text{OH}$  is less than 28%, add correspondingly larger volume and dissolve the citric acid in correspondingly smaller volume of  $\text{H}_2\text{O}$ . Cool, and make exactly neutral as follows:

Transfer 10 ml of the citrate soln to one of standard test tubes of a hydrogen-ion comparator set with color standards and add 0.5 ml of 0.02% soln of phenol red or sufficient volume to give same concentration of indicator as used in color standards. Add from graduated pipet a few drops of  $\text{NH}_4\text{OH}$  (1 + 7), mix, compare color by use of comparator with that of color standards of same indicator, add more  $\text{NH}_4\text{OH}$ , if necessary, and repeat test until color matches that of color standard corresponding to pH of 7.0. If the  $\text{NH}_4\text{OH}$  added is in excess of that required to give pH of 7.0,

\* If the sample is of such nature that it will not give a precipitate with  $\text{NH}_4\text{OH}$  as a test of neutralization, make the soln slightly alkaline to litmus with  $\text{NH}_3$  and then slightly acid with dilute  $\text{HNO}_3$ .

repeat test, using smaller quantity of  $\text{NH}_4\text{OH}$ . From quantity of  $\text{NH}_4\text{OH}$  required to produce in sample a color that exactly matches standard, calculate quantity of  $\text{NH}_4\text{OH}$  required to neutralize soln. Add this quantity of  $\text{NH}_4\text{OH}$ , check pH of soln by repeating test as before with the addition of a small quantity of  $\text{NH}_4\text{OH}$  or of a citric acid soln as may be required. When color matches, dilute soln, if necessary, to density of 1.09 at  $20^\circ$ . (Volume will be ca 2 liters.) Keep in tightly stoppered bottles and check pH from time to time.

Phenol red is recommended in place of bromothymol blue as salt effect due to presence of  $\text{NH}_4$  citrate soln gives pH reading with latter indicator that is ca 0.20 unit too high to obtain true reading. When bromothymol blue is used, subtract 0.20 from observed reading.

The other reagents and solns are described under 7 and 10.

## 16

## DETERMINATION

(a) *Acidulated samples*.—After washing out water-soluble  $\text{P}_2\text{O}_5$ , 13, transfer filter and residue, within period not to exceed an hour, to 250 ml flask containing 100 ml of the  $\text{NH}_4$  citrate soln previously heated to  $65^\circ$  in water bath. Close flask tightly with a smooth rubber stopper and shake vigorously until filter paper is reduced to a pulp, relieving pressure by momentarily removing stopper. Loosely stopper flask to prevent evaporation and return it to the bath. Maintain contents of flask at exactly  $65^\circ$ , keeping level of  $\text{H}_2\text{O}$  in bath above that of the citrate soln in flask. Shake flask every 5 min. At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath and immediately filter contents as rapidly as possible thru Whatman filter paper No. 5 or other paper of equal speed and retentiveness. (It is recommended that filtration be made with suction and use of Büchner funnel or ordinary glass funnel with Pt or other cone.) Wash with  $\text{H}_2\text{O}$  at  $65^\circ$  until volume of filtrate is ca 350 ml, allowing time for thoro draining before adding new portions of  $\text{H}_2\text{O}$ . If sample gives a cloudy filtrate, wash with a 5% soln of  $\text{NH}_4\text{NO}_3$ . Determine  $\text{P}_2\text{O}_5$  in citrate-insoluble residue by one of following methods: (1) Dry filter and contents, transfer to crucible, ignite until all organic matter is destroyed, and digest with 10–15 ml of  $\text{HCl}$  until all phosphate is dissolved; (2) transfer wet filter with contents to 200 ml flask, add 30–35 ml of  $\text{HNO}_3$  and 5–10 ml of  $\text{HCl}$ , and boil until all phosphate is dissolved; or (3) treat filter and contents as directed under 8(c) or (d). Dilute soln to 200 ml, mix well, filter thru a dry filter, and proceed as directed under 9 or 12.

(b) *Non-acidulated samples other than basic slag*.—Place 1 g of sample on 9 cm filter paper. Without previous washing with  $\text{H}_2\text{O}$ , proceed as directed under (a) and determine  $\text{P}_2\text{O}_5$  as directed under 9 or 12. If substance contains much animal matter (bone, fish, etc.), dissolve residue insoluble in  $\text{NH}_4$  citrate by one of processes described under 8(c) or (d).

## 17

## CITRATE-SOLUBLE AND AVAILABLE PHOSPHORIC ACID—OFFICIAL

Subtract sum of water-soluble and citrate-insoluble  $\text{P}_2\text{O}_5$  from total to obtain the citrate-soluble  $\text{P}_2\text{O}_5$ . Subtract citrate-insoluble  $\text{P}_2\text{O}_5$  from total to obtain available  $\text{P}_2\text{O}_5$ .

## 18

## DETECTION OF NITRATES—OFFICIAL

Mix 5 g of fertilizer with 25 ml of hot  $\text{H}_2\text{O}$ , and filter. To 1 volume of this soln add 2 volumes of  $\text{H}_2\text{SO}_4$ , free from  $\text{HNO}_3$  and oxides of N, and allow mixture to cool. Add few drops of concentrated soln of ferrous sulfate in such manner that fluids will not mix. If nitrates are present, junction shows at first purple, afterwards

brown color, or if only minute quantity is present, a reddish color. To another portion of soln add 1 ml of a 1% soln of  $\text{NaNO}_3$  and test as before to determine whether sufficient  $\text{H}_2\text{SO}_4$  was added in first test.

## ORGANIC AND AMMONIACAL NITROGEN ONLY

19

## REAGENTS

For ordinary work 0.5 *N* acid is recommended, but in determining very small quantities of N, 0.1 *N* acid is recommended. In titrating mineral acids against soln of  $\text{NH}_4\text{OH}$  use cochineal or methyl red as indicator.

(a) *Standard hydrochloric acid*.—Proceed as directed under 5 and 6, XLII, or determine absolute strength as follows:

**PRELIMINARY TEST:** Place a measured portion of the acid in Erlenmeyer flask and add excess of  $\text{CaCO}_3$  to neutralize free acid and few drops of 10% soln of  $\text{K}_2\text{CrO}_4$  as indicator. Titrate with 0.1 *N*  $\text{AgNO}_3$  soln and note exact quantity required to precipitate the chlorides.

**FINAL DETERMINATION:** To a measured portion of the acid add from buret 1 drop in excess of required quantity of  $\text{AgNO}_3$  soln as determined by preliminary test. Heat mixture to boiling, protect from light, and allow to stand until precipitate is granular. Filter on Gooch crucible, previously heated to 140–150° and weighed; wash with hot  $\text{H}_2\text{O}$ , testing filtrate to verify an excess of  $\text{AgNO}_3$ . Dry the  $\text{AgCl}$  at 140–150°, cool, and weigh.

(b) *Standard sulfuric acid*.—The acids may be standardized by any of the official methods in XLII. Or, determine strength of acid by precipitation with  $\text{BaCl}_2$  soln as follows: Dilute measured quantity of the acid to ca 100 ml; heat to boiling; and add, dropwise, a 10% soln of  $\text{BaCl}_2$  until no further precipitation occurs. Continue boiling ca 5 min., allow to stand 5 hours or longer in a warm place, pour supernatant liquid on weighed Gooch crucible or ashless filter, treat precipitate with 25–30 ml of boiling  $\text{H}_2\text{O}$ , transfer to filter, and wash with boiling  $\text{H}_2\text{O}$  until filtrate is free from chlorides. Dry, ignite over a Bunsen burner, and weigh as  $\text{BaSO}_4$ .

(c) *Standard alkali soln*.—A 0.1 *N* soln is recommended. Accurately determine strength of this soln by titration against the standard acid prepared as directed under (a) or (b), or proceed as directed in any official method under XLII.<sup>a</sup>

(d) *Sulfuric acid*.—Should contain 93–96%  $\text{H}_2\text{SO}_4$  and be free from nitrates and  $(\text{NH}_4)_2\text{SO}_4$ .

(e) *Metallic mercury, or mercuric oxide*.— $\text{HgO}$  of reagent grade, free from N.

(f) *Sulfide, or thiosulfate soln*.—Dissolve 40 g of commercial  $\text{K}_2\text{S}$  in 1 liter of  $\text{H}_2\text{O}$ . A soln of 40 g of  $\text{Na}_2\text{S}$  or 80 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in a liter may be used.

(g) *Sodium hydroxide soln*.—Dissolve ca 450 g of commercial  $\text{NaOH}$ , free from nitrates, in 1 liter of  $\text{H}_2\text{O}$ . (A soln having sp. gr. of 1.36 or higher may be used.)

(h) *Cochineal indicator*.—Digest 3 g of pulverized cochineal in mixture of 50 ml of alcohol and 200 ml of  $\text{H}_2\text{O}$  1 or 2 days at ordinary temp., agitating frequently, and filter.

(i) *Methyl red indicator*.—Dissolve 1 g of methyl red in 200 ml of alcohol.

Test reagents before using by blank determination with sugar, which insures partial reduction of any nitrates present.

20

## APPARATUS

(a) *Kjeldahl flasks for digestion and distillation*.—Total capacity ca 550 or 800 ml. Made of hard, moderately thick, and well-annealed glass.

(b) *Distillation flask*.—Use any suitable flask of ca 550 or 800 ml capacity, fitted with rubber stopper thru which passes lower end of Kjeldahl connecting bulb to

prevent NaOH being carried over mechanically during distillation. Use bulb 5 or 6 cm in diameter, and connect upper end of bulb tube to condenser tube by means of rubber tubing.

21

*Kjeldahl Method—Official*

Place 0.7–3.5 g, according to N content of material to be analyzed, in a digestion flask. Add ca 0.7 g of HgO, or its equivalent in metallic Hg, and 20–30 ml of H<sub>2</sub>SO<sub>4</sub> (0.1–0.3 g of crystallized CuSO<sub>4</sub> may also be used in addition to the Hg, or in many cases, in place of it). Place flask in inclined position and heat below boiling point of acid until frothing has ceased. (A small piece of paraffin may be added to prevent extreme foaming.) Increase heat until acid boils briskly and digest for a time after mixture is colorless or nearly so, or until oxidation is complete (ca 2 hours).

After cooling, dilute with ca 200 ml of H<sub>2</sub>O, and add a few pieces of granulated Zn or pumice stone to prevent bumping, and 25 ml of the K<sub>2</sub>S or Na<sub>2</sub>S or Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln with shaking. (If Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> is to be used, it should first be mixed with the NaOH so that they may be added together. When no Hg or HgO is to be used the addition of K<sub>2</sub>S or Na<sub>2</sub>S or Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln is unnecessary.) Add sufficient NaOH soln to make reaction strongly alkaline (50 ml usually sufficient), pouring it down side of flask so that it does not mix at once with the acid soln. Connect flask to condenser by means of Kjeldahl connecting bulb, taking care that tip of condenser extends below surface of the standard acid in receiver; mix contents by shaking and distil until all NH<sub>3</sub> has passed over into a measured quantity of the standard acid. (First 150 ml of distillate generally contains all the NH<sub>3</sub>.) Titrate with standard alkali soln, using the methyl red or cochineal indicator.

22

*Gunning Method—Official*

Place 0.7–3.5 g, according to N content of material to be analyzed, in a digestion flask. Add 10 g of powdered K<sub>2</sub>SO<sub>4</sub> or anhydrous Na<sub>2</sub>SO<sub>4</sub> and 15–25 ml (ordinarily ca 20 ml) of H<sub>2</sub>SO<sub>4</sub> (0.1–0.3 g of crystallized CuSO<sub>4</sub> may also be added). Conduct digestion as in Kjeldahl process, starting with temp. below boiling point and increasing heat gradually until frothing ceases. Digest for a time after mixture is colorless or nearly so, or until oxidation is complete (usually 2 hours). Complete as directed under 21, but do not add K<sub>2</sub>S or Na<sub>2</sub>S or Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. In making mixture alkaline before distilling add litmus paper or a few drops of phenolphthalein indicator. (Pink color given by phenolphthalein, indicating an alkaline reaction, is destroyed by excess of strong fixed alkali.)

23

*Kjeldahl-Gunning-Arnold Method—Official*

Place 0.7–3.5 g, according to N content of material to be analyzed, in a digestion flask. Add 15–18 g of K<sub>2</sub>SO<sub>4</sub> or anhydrous Na<sub>2</sub>SO<sub>4</sub>, 1 g of CuSO<sub>4</sub> or ca 0.7 g of HgO (or its equivalent in metallic Hg), and 25 ml of the H<sub>2</sub>SO<sub>4</sub>. Heat mixture gently until frothing ceases, then boil briskly, and continue digestion for a time after mixture is colorless or nearly so, or until oxidation is complete (ca 2 hours). Cool, add ca 200 ml of H<sub>2</sub>O, and if HgO or metallic Hg has been used, add also 50 ml of the K<sub>2</sub>S or Na<sub>2</sub>S or Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln. Make strongly alkaline with the NaOH soln and proceed as directed under 21.

## TOTAL NITROGEN

*Kjeldahl Method Modified to Include Nitrogen of Nitrates—Official*

24

REAGENTS AND APPARATUS.—See 19 and 20.

25

## DETERMINATION

Place 0.7–3.5 g, according to N content of material to be analyzed, in Kjeldahl digestion flask. (1) Add 30 ml of the  $\text{H}_2\text{SO}_4$  containing 1 g of commercial salicylic acid, shake until *thoroly* mixed, allow to stand at least 30 min. with frequent shaking or until complete soln results, and then add 5 g of crystallized  $\text{Na}_2\text{S}_2\text{O}_3$  and digest as directed below; or, (2) add to substance 30 ml of  $\text{H}_2\text{SO}_4$  containing 2 g of the salicylic acid, allow to stand at least 30 min. with frequent shaking or until complete soln results, and then add gradually 2 g of Zn dust (an impalpable powder—granulated Zn or filings not satisfactory), shaking contents of flask at same time, and digest as follows:

Heat over low flame until all danger from frothing has passed. Increase heat until acid boils briskly and continue boiling until white fumes no longer escape from flask (5–10 min.). Add ca 0.7 g of  $\text{HgO}$ , or its equivalent in  $\text{Hg}$ , and continue boiling until liquid in flask is colorless, or nearly so. If contents of flask are likely to become solid before this point is reached, add 10 ml more of  $\text{H}_2\text{SO}_4$ . Complete determination as directed under 21. Test reagents by blank determinations.

*Gunning Method Modified to Include Nitrogen of Nitrates—Official*

26

REAGENTS AND APPARATUS.—See 19 and 20.

27

## DETERMINATION

Place 0.7–3.5 g, according to N content of material to be analyzed, in digestion flask; add 30 ml of  $\text{H}_2\text{SO}_4$  containing 1 g of commercial salicylic acid; shake until thoroly mixed; and allow to stand, shaking frequently, at least 30 min., or until complete soln results. Add 5 g of  $\text{Na}_2\text{S}_2\text{O}_3$  and heat soln 5 min.; cool, add 10 g of  $\text{K}_2\text{SO}_4$  or anhydrous  $\text{Na}_2\text{SO}_4$ , heat very gently until foaming ceases, and proceed with digestion as directed under 22.

## AMMONIACAL NITROGEN

28

*Magnesium Oxide Method—Official*

Place 0.7–3.5 g, according to  $\text{NH}_3$  content of material to be analyzed, in a distillation flask with ca 200 ml of  $\text{H}_2\text{O}$  and 2 g or more of  $\text{MgO}$  free from carbonates. Connect flask to condenser by means of Kjeldahl connecting bulb, distil 100 ml of liquid into measured quantity of standard acid, and titrate with standard alkali, using cochineal or methyl red indicator, 19(h) or (i).

## NITRATE AND AMMONIACAL NITROGEN

29

*Ferrous Sulfate-Zinc-Soda Method—Official*

(Not applicable in presence of organic matter, calcium cyanamide, and urea.)

Place 0.35, 0.5, or 0.7 g of sample in 600–700 ml flask and add 200 ml of  $\text{H}_2\text{O}$ , 5 g of powdered Zn, 1–2 g of ferrous sulfate, and 50 ml of  $\text{NaOH}$  soln (sp. gr. 1.33). Connect flask with the distilling apparatus, distil, collect distillate in usual way in 0.1 N  $\text{H}_2\text{SO}_4$ , and titrate with standard alkali, using cochineal or methyl red indicator, 19(h) or (i).

30

*Devarda Method<sup>7</sup>—Official*

(Not applicable in presence of organic matter, calcium cyanamide, and urea.)

Place 0.5 g of sample in a 600–700 ml flask and add 300 ml of  $\text{H}_2\text{O}$ , 3 g of Devarda

alloy and 5 ml of NaOH soln (42% by weight), pouring latter down side of flask so that it does not mix at once with contents. Connect, by means of Daviss<sup>8</sup> or other suitable scrubbing bulb that will prevent passing over of any portion of spray, with condenser, the tip of which always extends beneath surface of standard acid in receiving flask. Mix contents of distilling flask by rotating. Heat slowly at first and then at such a rate that the 250 ml of distillate required will pass over in 1 hour. Collect distillate in measured quantity of standard acid, 19(a) or (b), and titrate with standard alkali soln, 19(c), using cochineal or methyl red indicator, 19(h) or (i).

In the analysis of nitrate salts, proceed as directed above but use 25 ml of the nitrate soln equivalent to 0.50 g of the sample.

#### NITRATE NITROGEN

31

##### *Robertson Method<sup>9</sup>—Official*

(Applicable in presence of calcium cyanamide and urea.)

(a) Determine total N as directed under 25 or 27.

(b) Weigh out 2.0 g of the fertilizer mixture on 11 cm Whatman No. 2 filter paper, wash with H<sub>2</sub>O to nearly 200 ml in graduated flask, and make up to volume. If mixture is greasy or does not wet easily, moisten sample with 7 ml of alcohol and continue washing to nearly 200 ml. Determine N in residue as directed under 21, 22, or 23.

(c) Determine ammoniacal N in 50 ml of filtrate as directed under 28.

(d) Place another 50 ml portion of filtrate in 500 ml Kjeldahl flask and add 2 g of ferrous sulfate and 20 ml of H<sub>2</sub>SO<sub>4</sub>. (If total N is over 5%, use 5 g of ferrous sulfate.) Digest over hot flame until all H<sub>2</sub>O is evaporated and white fumes appear and continue digestion at least 10 min. to drive off nitrate N. If severe bumping occurs, add 10–15 glass beads. Add 0.65 g of Hg or its equivalent of HgO and digest until all the organic matter is oxidized. Cool, dilute, add the K<sub>2</sub>S soln, and proceed as directed under 25. Add a pinch of mixture of Zn dust and granular Zn (20-mesh) to each flask before distillation to prevent bumping.

Total N(a) – water-insoluble N(b) = water-soluble N. Water-soluble N – N obtained in (d) = nitrate N.

Ammoniacal N + nitrate N = mineral N. Total N – mineral N = organic N.

32

##### *Jones Modification of Robertson Method<sup>10</sup>—Official*

(Applicable when determination of water-soluble nitrogen is not needed.)

Weigh 0.5 g of sample into Kjeldahl flask, add 50 ml of H<sub>2</sub>O, and rotate gently. Add 2 g of ferrous sulfate and rotate. Add 20 ml of H<sub>2</sub>SO<sub>4</sub>. Digest over hot flame. When H<sub>2</sub>O is evaporated and white fumes appear, add 0.65 g of Hg and proceed as directed under 21. Cool, dilute, and distil as usual. Total N – N thus found = nitrate N.

33

#### WATER-INSOLUBLE NITROGEN IN CYANAMIDE<sup>11</sup>—OFFICIAL

Weigh 2 g of finely ground cyanamide and place in mortar. Gradually add ca 70 ml of H<sub>2</sub>O while stirring with pestle and grind thoroly. Transfer mixture to beaker, washing out mortar with H<sub>2</sub>O. Filter on 11 cm paper. When all cyanamide has been transferred to the paper, wash with an additional 250 ml of H<sub>2</sub>O, allowing time for complete drainage before adding more H<sub>2</sub>O. Remove filter paper and residue to digestion flask. Determine insoluble nitrogen in residue as directed under 21, 22, or 23.

## NITROGEN ACTIVITY

34

*Water-Insoluble Organic Nitrogen—Official*

Place 1 or 1.4 g of the material on 11 cm filter paper wet with alcohol, and wash with  $\text{H}_2\text{O}$  at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with  $\text{H}_2\text{O}$ , wash with 5 ml of alcohol and then with requisite quantity of  $\text{H}_2\text{O}$ . Dry, and determine N in residue as directed under 21 or 22, making correction for water-insoluble N of filter paper, if necessary.

35

*Beaker Method—Official, first action*

Place 1 or 1.4 g of the material in a 50 ml beaker, wet with alcohol, add 20 ml of  $\text{H}_2\text{O}$ , and allow to stand 15 min., with occasional stirring. Transfer supernatant liquid to 11 cm. Whatman No. 2 filter paper, and wash residue 4 or 5 times by decantation with  $\text{H}_2\text{O}$  at room temp. (20–25°). Use long-stemmed funnels 2.5" in diameter and having an angle of 60°. Finally transfer all residue to filter paper and complete washing until filtrate measures 250 ml. Dry, and determine N in residue as directed under 21 or 22.

36

*Removal of Water-Soluble Nitrogen—Official*

(a) *Mixed fertilizers*.—Place the quantity of material equivalent to 50 mg of water-insoluble organic N, 34, on 11 cm filter paper wet with alcohol, and wash with  $\text{H}_2\text{O}$  at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with  $\text{H}_2\text{O}$ , wash with 5 ml of alcohol and then with requisite quantity of  $\text{H}_2\text{O}$ . If necessary to use 4 g or more of the material, weigh required quantity into a small beaker, wet with alcohol, wash by decantation, finally transfer to filter, and finish extraction as directed previously.

(b) *Raw materials*.—Place the quantity of material equivalent to 50 mg of water-insoluble N, 34, in small mortar; add ca 2 g of powdered rock phosphate, mix thoroly, transfer to filter paper wet with alcohol, and wash with  $\text{H}_2\text{O}$  at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with  $\text{H}_2\text{O}$ , wash with 5 ml of alcohol and then with requisite quantity of  $\text{H}_2\text{O}$ .

37 *Water-Insoluble Organic Nitrogen Soluble in Neutral Permanganate—Official*

Using 25 ml of tepid  $\text{H}_2\text{O}$ , transfer insoluble residue obtained in 36 to 400 ml Griffin low-form beaker; add 1 g of  $\text{Na}_2\text{CO}_3$ , mix, and add 100 ml of 2% soln of  $\text{KMnO}_4$ . Cover with watch-glass and immerse 30 min. in steam or hot water bath, keeping liquid in beaker below that of  $\text{H}_2\text{O}$  in bath. Stir twice at intervals of 10 min. At end of 30 min. remove from bath, add immediately 100 ml of cold  $\text{H}_2\text{O}$ , and filter thru heavy 15 cm folded filter. Wash with small quantities of cold  $\text{H}_2\text{O}$  until filtrate measures ca 400 ml. Determine N in residue and filter as directed under 21 or 22, correcting for N contained in filter. The N thus obtained is the inactive water-insoluble organic N. The N obtained under 34—percentage of N found = water-insoluble organic N soluble in neutral permanganate.

*Water-Insoluble Organic Nitrogen Distilled from Alkaline Permanganate<sup>12</sup>—Official*

38

## REAGENTS

(a) *Stock soln of potassium permanganate*.—Dissolve 50 g of  $\text{KMnO}_4$  in liter of  $\text{H}_2\text{O}$ . Dissolve 0.5 g of Na oxalate in 300 ml of  $\text{H}_2\text{O}$  and 10 ml of  $\text{H}_2\text{SO}_4$ . Heat to



75–80° and titrate with the  $\text{KMnO}_4$  soln, using a Mohr pipet or an all-glass buret to contain permanganate soln.  $235.89 \div \text{result of titration in ml} = \text{concentration of } \text{KMnO}_4 \text{ in g per liter}$ . Adjust concentration to 50 g per liter, protect from light, and store at a temp. above 15°.

(b) *Stock soln of sodium hydroxide*.—Dissolve 300 g of NaOH in 1 liter of  $\text{H}_2\text{O}$ . Cool before using.

(c) *Alkaline permanganate soln*.—Mix equal quantities of stock solns (a) and (b) and add 10 ml of  $\text{H}_2\text{O}$  for each liter of soln that mixture is calculated to make. Use this soln immediately, as it is unstable.

## 39

## DETERMINATION

Dry residue remaining after treatment of material as directed in 36 at temp. not exceeding 80° and transfer from filter to 500–600 ml Kjeldahl distillation flask, loosening adhering particles by rubbing gently with stiff brush but avoiding transfer of portions of brush or of paper fibers. Add 20 ml of  $\text{H}_2\text{O}$ , 15–20 small glass beads or fragments of pumice stone, a drop of mineral lubricating oil weighing not more than 50 mg, and 100 ml of the alkaline permanganate soln. Connect with upright condenser to lower end of which has been attached 100 ml graduated cylinder containing standard acid and so arranged as to receive distillate below surface of acid or otherwise trapped so as to prevent loss of  $\text{NH}_3$  fumes. Digest slowly with very low flame 30 min., barely below distillation point, using coarse wire gauze and asbestos paper between flask and flame. Gradually raise temp., and after all danger from frothing has passed distil 95 ml in 60 min. ( $\pm 5$  min.), controlling distillation to obtain ca 24 ml of distillate in each 15 min. period. Conduct first part of distillation over bare flame but use wire gauze 10 min. before completion to avoid breaking flask. Transfer distillate to Erlenmeyer flask or to beaker and titrate with standard alkali, using cochineal or methyl red indicator. When a tendency to froth is noticed, lengthen digestion period, and no trouble will be experienced when distillation is begun. During digestion gently rotate flask occasionally, particularly if material shows tendency to adhere to sides.

The N thus obtained is active water-insoluble organic N. If it is found to be less than 55% of the total water-insoluble organic N present, it is recommended that a second portion of the sample be prepared as directed under 36. Dry residue below 80°, transfer from filter to Kjeldahl flask as directed above, and determine N as directed under 21 or 22. Recalculate percentage of active water-insoluble N on basis of quantity of water-insoluble N thus found.

Previous to digestion with alkaline permanganate, the washed sample may be transferred from filter to flask by spreading filter on a metal disk bent to form a trough that fits the palm of the hand, brushing larger portion of material into flask with spatula, and washing in remainder with 20 ml of  $\text{H}_2\text{O}$  from 20 ml pipet or small wash bottle. Do not add more  $\text{H}_2\text{O}$  before digestion with alkaline permanganate, but, with this exception, proceed as with transfer of dried material.

## POTASH

*Method I. Lindo-Gladding*<sup>13</sup>—Official

## 40

## REAGENTS

(a) *Ammonium chloride soln*.—Dissolve 100 g of  $\text{NH}_4\text{Cl}$  in 500 ml of  $\text{H}_2\text{O}$ , add 5–10 g of pulverized  $\text{K}_2\text{PtCl}_6$ , and shake at intervals 6–8 hours. Allow mixture to settle overnight and filter. (The residue may be used for preparation of a fresh supply.)

(b) *Platinum soln.*—Use a Pt soln containing the equivalent of 1 g of Pt (2.1 g of  $\text{H}_2\text{PtCl}_6$ ) or a Pt soln containing the equivalent of 0.5 g of Pt (1.05 g  $\text{H}_2\text{PtCl}_6$ ) in every 10 ml. For materials containing less than 15% of  $\text{K}_2\text{O}$ , a Pt soln containing 0.2 g of metallic Pt (0.42 g of  $\text{H}_2\text{PtCl}_6$ ) in each 10 ml is recommended.

(c) *Diglycol stearate soln.*—Dissolve 20 g of diglycol stearate Tech. in 1 liter of equal parts of benzene and ethyl alcohol.

## 41

## PREPARATION OF SOLUTION

(a) *Mixed fertilizers.*—Place 2.5 g of sample in a 250 ml volumetric flask, and add 125 ml of  $\text{H}_2\text{O}$  and 50 ml of saturated  $\text{NH}_4$  oxalate soln, also 1 ml of the diglycol stearate when necessary to prevent foaming. Boil 30 min., add slight excess of  $\text{NH}_4\text{OH}$ , and after cooling dilute to 250 ml, mix, and pass thru dry filter.

(b) *Potash salts; muriate and sulfate of potash, sulfate of potash and magnesia; and kainit.*—Dissolve 2.5 g and dilute to 250 ml without addition of  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate. When substances that interfere, such as ammonia, lime, aluminum, etc., are present, proceed as directed in (a).

(c) *Organic compounds (cottonseed meal, tobacco stems, etc.).*—For total  $\text{K}_2\text{O}$  saturate 10 g of sample with  $\text{H}_2\text{SO}_4$  and ignite in muffle at *low red heat* to destroy organic matter. Add a little  $\text{HCl}$ , warm slightly in order to loosen mass from dish, transfer to 500 ml volumetric flask, add  $\text{NH}_4\text{OH}$  and saturated  $\text{NH}_4$  oxalate soln, cool, dilute to 500 ml, mix, pass thru a dry filter, and proceed as directed under 42(a).

(d) *Ashes from wood, cotton hulls, etc.*—Boil 10 g of sample with 300 ml of  $\text{H}_2\text{O}$  30 min., and add to hot soln a slight excess of  $\text{NH}_4\text{OH}$  and then sufficient saturated  $\text{NH}_4$  oxalate soln to precipitate all lime present. Cool, dilute to 500 ml, mix, and pass thru dry filter.

## 42

## DETERMINATION

(a) *Mixed fertilizers.*—Evaporate nearly to dryness a 25 or 50 ml aliquot of soln, 41(a), to which has been added sufficient potash-free normal  $\text{NaOH}$  (1–2 ml) to prevent formation of free phosphoric acid during ignition; add 1 ml of  $\text{H}_2\text{SO}_4$  (1+1) and 6–8 granules of granulated sugar, evaporate to dryness, and ignite to whiteness at low temp. Maintain a dull red heat until residue is perfectly white. Dissolve residue in hot  $\text{H}_2\text{O}$ , using at least 20 ml for each decigram of  $\text{K}_2\text{O}$  present, and add a few drops of  $\text{HCl}$  and then an excess of Pt soln. Evaporate on water bath to thick paste, avoiding exposure to  $\text{NH}_3$ . Treat residue with ca 6 ml of 80% alcohol, adding 0.6 ml of  $\text{HCl}$ . After 15 min. filter on Gooch crucible and wash precipitate thoroly with 80% alcohol, both by decantation and on filter, continuing washing after filtrate is colorless. Wash 5 or 6 times with 10 ml portions of the  $\text{NH}_4\text{Cl}$  soln to remove impurities from precipitate. Wash again thoroly with 80% alcohol and dry precipitate 30 min. at  $100^\circ$ . Weigh and calculate to  $\text{K}_2\text{O}$ . (Precipitate should be completely soluble in  $\text{H}_2\text{O}$ .)

(b) *Muriate of potash.*—Acidify 50 ml of the soln prepared according to 41(b) with few drops of  $\text{HCl}$ , add 10 ml of Pt soln, and evaporate to thick paste. Treat residue as directed under (a). If  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate are used in preparation of this soln, ignite and complete determination as directed under (a).

(c) *Sulfate of potash, sulfate of potash and magnesia, and kainit.*—Acidify 50 ml of the soln prepared according to 41(b) with a few drops of  $\text{HCl}$  and add 15 ml of the Pt soln. Evaporate mixture and proceed as directed under (a), but use 25 ml portions of the  $\text{NH}_4\text{Cl}$  soln. If  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate are used in preparation of this soln, ignite and complete determination as directed under (a) but use 25 ml portions of  $\text{NH}_4\text{Cl}$  soln.

(d) *Ashes from wood, cotton hulls, etc.*—Prepare soln according to 41(d) and proceed as directed under (a) paying special attention to last sentence.

For conversion of  $K_2PtCl_6$  to KCl use the factor 0.3061; to  $K_2SO_4$ , 0.35843; to  $K_2O$ , 0.19376.

#### WATER-SOLUBLE BORON<sup>14</sup>—OFFICIAL

43

#### REAGENTS

(a) *Standard sodium hydroxide soln.*—Prepare this soln free from carbonates by first making saturated soln (100 g of NaOH in 100 ml of  $H_2O$ ) in order to precipitate any  $Na_2CO_3$  present when soln is allowed to stand in vessel from which the  $CO_2$  of the air is excluded. Filter thru hard filter that has been soaked in alcohol, dilute portion with boiled and cooled  $H_2O$  to ca 0.1 *N*, and accurately determine strength of soln by titration, as directed under 44(a), against 0.1 *N* boric acid soln.

(b) *Methyl red indicator.*—Dissolve 0.1 g of methyl red in 50 ml of alcohol, dilute to 100 ml with  $H_2O$ , and filter if necessary.

44

#### DETERMINATION

(a) *Mineral salts.*—Dissolve 5–10 g of sample in 50–75 ml of hot  $H_2O$ , decomposing carbonates, if present, with a slight excess of HCl; heat to boiling and add sufficient 10%  $BaCl_2 \cdot 2H_2O$  soln to precipitate sulfates, using ca 10 ml in excess. Add in small quantities sufficient powdered  $Ba(OH)_2$  to make soln alkaline, avoiding large excess; and boil ca 5 min., or until any  $NH_3$  present has been expelled. Filter into 300 ml flask, wash residue, and make filtrate acid with HCl, using an excess equivalent to a few ml of 0.1 *N* soln. Boil 15 min. to expel  $CO_2$ , cool by placing flask in cold  $H_2O$ , and bring to neutrality by first adding 4 or 5 drops of the methyl red indicator and then the standard NaOH soln until color of soln changes from pink to yellow. If neutral point has been exceeded, or if there is any doubt as to this, restore pink color by adding a few drops of 0.1 *N* HCl and change color to yellow again with minimum quantity of the standard NaOH soln. Add 1–2 g of neutral mannitol (mannite) and a few tenths of a ml of phenolphthalein indicator, 10(d), note buret reading, and again titrate soln with the standard NaOH soln until pink color develops. Add a little more mannitol and if pink color disappears continue addition of standard alkali until it reappears. Repeat procedure until addition of mannitol has no further action on end point. (If content of boron in soln titrated is low, one addition of mannitol is usually sufficient.) From volume of standard alkali required in titration after addition of mannitol, corrected for volume required when running a blank, calculate quantity of boron in sample. 1 ml of 0.1 *N* NaOH soln = .00108 g of boron.

When an acid soln of sample gives no precipitate upon addition of a soln of  $CaCl_2$  and sufficient  $NH_4OH$  to give alkaline reaction, phosphates and Fe and Al salts are absent, and that portion of determination that involves treatment with  $BaCl_2$  and  $Ba(OH)_2$  for removal of these constituents may be omitted.

(b) *Mixed fertilizers and organic compounds.*—Weigh 5 g of sample into 250 ml beaker, add 50 ml of hot  $H_2O$ , cover with watch-glass, digest 15–20 min. on water bath, filter, and wash into another beaker of same capacity. Heat filtrate to boiling and add 15 ml of 10%  $BaCl_2 \cdot 2H_2O$  soln followed without undue loss of time by sufficient powdered  $Ba(OH)_2$  to give an alkaline reaction as indicated by phenolphthalein; boil ca 5 min. (gently to prevent frothing over), filter, and wash. Or, if preferred, make up to mark in a volumetric flask and take an aliquot. Evaporate filtrate or aliquot to dryness in Pt or porcelain dish and ignite residue (preferably in muffle furnace) just below redness, until organic matter is completely carbonized. Treat

ignited residue with hot  $\text{H}_2\text{O}$ , make slightly acid with  $\text{HCl}$ , heat nearly to boiling. make alkaline again with a slight excess of  $\text{Ba}(\text{OH})_2$ , and filter into 300 ml flask. Acidify with  $\text{HCl}$  (1+9), using excess equivalent to few ml of a 0.1  $N$  soln, boil to expel  $\text{CO}_2$ , and titrate as directed under (a).

If the  $\text{Ba}(\text{OH})_2$  has been added only in slight excess there is tendency for filtrate to become acid during evaporation with possible loss of boron. It is important, therefore, that soln be kept alkaline, by repeated additions of  $\text{Ba}(\text{OH})_2$ , if necessary, until evaporation is completed.

If filtrate from the  $\text{BaCl}_2\text{--Ba}(\text{OH})_2$  precipitate is titrated before soluble organic matter is destroyed, the end points in the titration will usually be too indefinite to give accurate results. The purpose in evaporating filtrate and igniting residue, therefore, is to get rid of the soluble organic constituents that interfere with titration. When sample contains relatively high boron content (in excess of 0.5%) a smaller sample may be taken and the quantity of organic matter present may then be too small to interfere seriously with sharpness of the end points during titration. When such is the case, boil the soln after addition of the  $\text{Ba}(\text{OH})_2$  until any  $\text{NH}_3$  present has been expelled. Omit evaporating filtrate from  $\text{BaCl}_2\text{--Ba}(\text{OH})_2$  precipitate. Add to filtrate an excess of  $\text{HCl}$  equivalent to a few ml of a 0.1  $N$  soln, boil to expel  $\text{CO}_2$ , and titrate as directed under (a).

#### ACID-SOLUBLE BORON<sup>15</sup>—OFFICIAL

45

##### APPARATUS

The apparatus<sup>16</sup> consists of two 200 ml round-bottomed flasks, Liebig condenser, and 200 ml Erlenmeyer receiving flask. One of the round-bottomed flasks, No. 2, has a rubber stopper with two holes. Thru one hole passes a glass tube running to bottom of flask; thru other hole passes a short tube leading to condenser. The other flask, No. 1, is fitted with perforated rubber stopper and short bent tube connected by rubber tubing with the long tube in flask No. 2. The whole apparatus is supported by clamps and rings on two stands.

46

##### DETERMINATION

If the material to be examined is a mixed fertilizer or contains less than the equivalent of 2% of anhydrous borax, weigh 5 g into flask No. 2; if the material contains much more than 2%, use 2 g. Add 5 ml of 85%  $\text{H}_3\text{PO}_4$  and 20 ml of methyl alcohol and connect flask with condenser. Add 100 ml of methyl alcohol (at least 95%) to flask No. 1, which is set in water bath and connected with flask No. 2. Place receiving flask in position at end of condenser and apply sufficient heat to water bath to keep a steady flow of bubbles of the methyl alcohol passing thru flask No. 2. Also apply some heat to flask No. 2 to keep volume at ca 25 ml. Continue distillation until 100 ml of distillate is obtained (ca 30 min.). Add to the distillate 2 or 3 drops of phenolphthalein indicator, 10(d), and 5–10 ml of 0.1  $N$   $\text{NaOH}$ , or enough to produce a permanent pink color. Stopper flask, shake well, and connect at once with condenser by means of Hopkins or similar bulb. Using water bath (not gas burner), distil the alcohol and use for another determination. Transfer residue, which should be not less than 10 ml, to Pt or porcelain dish, using as little  $\text{H}_2\text{O}$  as possible, and evaporate to dryness on steam or water bath. When dry, ignite below redness, acidify with few drops of ca 1  $N$   $\text{HCl}$ , add 20–25 ml of  $\text{H}_2\text{O}$ , and warm 1–2 min. on steam bath. Filter into small flask, thoroly wash, dilute to 50–75 ml, attach to an air-cooled condenser, and boil gently for few minutes to remove  $\text{CO}_2$ . Add 3 or 4 drops of methyl red indicator, 43(b), and then 0.1  $N$   $\text{NaOH}$  until red color just disappears. Add ca 1 g of mannitol, or less if but a small amount of boron is present. (At this point, if boron is present, soln will take on pinkish color; depth of color depends on quantity present, 0.01 or 0.02% usually being sufficient to

give the color if soln has been carefully neutralized with the NaOH soln.) Add 2 or 3 drops of the phenolphthalein indicator and titrate with the 0.1 *N* NaOH. Test reagents by blank determination. (If the NaOH is free from CO<sub>2</sub> the blank should not be more than 0.2 ml.) Calculate to boron as directed under 44(a).

47

## ACID-SOLUBLE CALCIUM—TENTATIVE

Proceed as directed under XXVII, 44, using bromophenol blue as indicator.

## WATER-SOLUBLE CHLORINE—OFFICIAL

48

## REAGENTS

(a) *Standard silver nitrate soln.*—Dissolve ca 5 g of pure recrystallized AgNO<sub>3</sub> in H<sub>2</sub>O and dilute to 1 liter. Standardize against pure, dry NaCl and adjust so that 1 ml of soln = 0.001 g of Cl.

(b) *Potassium chromate indicator.*—Dissolve 5 g of K<sub>2</sub>CrO<sub>4</sub> in 100 ml of H<sub>2</sub>O.

49

## DETERMINATION

Place 2.5 g of sample on 11 cm filter paper and wash with successive portions of boiling H<sub>2</sub>O until the washings amount to nearly 250 ml, collecting the filtrate in 250 ml volumetric flask. Cool, dilute to mark with H<sub>2</sub>O, and mix well. Pipet 50 ml into 150 ml beaker, add 1 ml of the K<sub>2</sub>CrO<sub>4</sub> indicator, and titrate with the AgNO<sub>3</sub> soln until color produced by Ag<sub>2</sub>CrO<sub>4</sub> appears as permanent red.

## COPPER—TENTATIVE

50

## REAGENTS

(a) *Wash soln.*—Add 5 ml H<sub>2</sub>SO<sub>4</sub> to 995 ml of H<sub>2</sub>O.

(b) *Sodium thiosulfate soln.*—Dissolve 7.8 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · 5H<sub>2</sub>O in H<sub>2</sub>O and dilute to 1 liter. This soln is approximately equivalent to 0.002 g of Cu per ml.

(c) *Copper nitrate soln.*—Dissolve 2.00 g of pure Cu (electrolytic) in an excess of HNO<sub>3</sub> and dilute to 1 liter with H<sub>2</sub>O. This soln is equivalent to 0.002 g of Cu per ml.

(d) *Starch indicator soln.*—Mix ca 1 g of soluble starch with enough cold H<sub>2</sub>O to make a thin paste; add ca 100 ml of boiling H<sub>2</sub>O and boil while stirring ca 1 min.

51

## DETERMINATION

To 2.5 g of sample in a Kjeldahl flask add ca 10 ml of HNO<sub>3</sub> and exactly 10 ml of H<sub>2</sub>SO<sub>4</sub>. Boil down to white fumes. If soln becomes dark due to organic matter add a little more HNO<sub>3</sub> and boil down again to white fumes, repeating if necessary until the organic matter is destroyed. Cool, and add 100 ml of H<sub>2</sub>O. Boil 3–5 min. and cool to room temp. Filter with suction thru a mat of filter paper pulp. Wash out the flask and wash filter at least 5 times with the wash soln. Dilute filtrate to 250 ml with H<sub>2</sub>O in a volumetric flask.

Pipet 100 ml of the prepared soln into 250 ml Erlenmeyer flask. Pass H<sub>2</sub>S thru soln ca 15 min. Saturate some of the wash soln in a wash bottle with H<sub>2</sub>S (ca 50 ml of soln for each sample). Filter sample soln thru 11 cm No. 5 Whatman or similar paper. Wash out flask and inlet tube, both inside and out, with the H<sub>2</sub>S wash soln and wash precipitate seven more times with small portions of this soln, keeping filter funnel covered with watch-glass as much of the time as possible.

Place paper and precipitate in glazed porcelain crucible and ignite at low temp. until the C is completely oxidized. Wash down the H<sub>2</sub>S delivery tube, both inside

and out, into flask in which precipitation was made, first with bromine  $\text{H}_2\text{O}$ , then with  $\text{HNO}_3$  (3+97). Add 1–2 ml of  $\text{HNO}_3$  to the Cu in the crucible and allow to stand a few minutes. Wash out crucible into flask with  $\text{HNO}_3$  (3+97). Add ca 5 ml of  $\text{HNO}_3$ . If the sample contains less than 0.01 g of Cu, add a measured amount of the  $\text{Cu}(\text{NO}_3)_2$  to bring the total Cu to 0.01 g or slightly more. To another 250 ml Erlenmeyer flask add as much of the  $\text{Cu}(\text{NO}_3)_2$  as is equivalent to the sample containing the most Cu or slightly more, then 5 ml of  $\text{HNO}_3$  and 2 or 3 ml of bromine  $\text{H}_2\text{O}$ . Hereafter treat all solns alike. Gently boil down these solns to ca 5 ml. If all the Cu has not dissolved, add more  $\text{HNO}_3$  and boil down again to 5 ml, and repeat if necessary. Do not evaporate to dryness. Add 50 ml of  $\text{H}_2\text{O}$  and boil ca 5 min. Cool, and add  $\text{NH}_4\text{OH}$  from a dropper until just one drop causes the change in color from the light blue of the cupric ion to the deep blue of the cupric ammonia ion. Avoid an excess. Add ca 5 ml of acetic acid and cool to room temp. Add 3–4 g of small crystals of KI (conveniently measured with spoon), stir until crystals have dissolved, and titrate immediately with the  $\text{Na}_2\text{S}_2\text{O}_3$  until the sample soln is light yellow color. Add ca 1 ml of the starch indicator and continue titration to disappearance of the starch-iodine color. Calculate Cu equivalent of thio-sulfate soln from titration of the soln containing the known amount of Cu and from this factor calculate amount of Cu in the sample soln. If additional  $\text{Cu}(\text{NO}_3)_2$  was added to the sample soln, subtract amount of Cu added from that found by titration. Report as Cu.

ACID-SOLUBLE MAGNESIUM<sup>18</sup>

52

*Method I—Official*

Weigh 2 g of sample into 200 ml volumetric flask, add 10 ml of  $\text{HCl}$  and 30 ml of  $\text{HNO}_3$ , boil gently 30 min., cool, and dilute to volume with  $\text{H}_2\text{O}$ . Pipet an aliquot containing not more than 18 mg of Mg into 250 ml beaker, add 6 ml of  $\text{H}_2\text{SO}_4$  (1+1), remove the cover, and evaporate until white fumes appear. Cool slightly, wash down inside surface of beaker with jet of  $\text{H}_2\text{O}$ , and again evaporate until fumes of  $\text{H}_2\text{SO}_4$  appear. Cool, add 10 ml of  $\text{H}_2\text{O}$ , stir thoroly, and digest on steam bath 10–15 min. Remove flask from steam bath, add 100 ml of alcohol, stir so that the  $\text{CaSO}_4$  is well dispersed thruout liquid, and allow to stand 2 hours or longer. Filter by means of suction thru tight plug of filter paper pulp, using Gooch crucible, and wash 5 times with 5 ml portions of alcohol containing 1 ml of  $\text{H}_2\text{SO}_4$  per 100 ml.

Evaporate alcoholic filtrate as far as possible on steam bath. Transfer soln to 250 ml Erlenmeyer flask, dilute to 75–100 ml, and add 2 g of citric acid and 10 ml of 25% soln of  $(\text{NH}_4)_2\text{HPO}_4$ . Add  $\text{NH}_4\text{OH}$  until soln is alkaline to litmus and then add 10 ml in excess. Add 5–10 glass beads, tightly stopper flask, and shake on machine at least 1 hour. Allow to stand in cool place 4 hours, or preferably overnight. Filter thru tight paper containing a little paper pulp, and wash with  $\text{NH}_4\text{OH}$  (5+95), containing 50 g of  $(\text{NH}_4)_2\text{HPO}_4$  per liter, until precipitate and paper are free from Fe and Al. Pass 25 ml of hot  $\text{HCl}$  (5+95) thru paper into flask, transfer soln to 250 ml beaker, and wash paper and flask thoroly with more of the diluted acid. To the soln in a volume of 50–75 ml and containing no glass beads, add 0.5 ml of a 25% soln of  $(\text{NH}_4)_2\text{HPO}_4$ , cool, and then add  $\text{NH}_4\text{OH}$  slowly and with stirring until soln is alkaline to litmus. Stir few minutes, add 3–4 ml of  $\text{NH}_4\text{OH}$ , and allow to stand 4 hours or overnight. Transfer precipitate to small filter or filtering crucible and wash with  $\text{NH}_4\text{OH}$  (5+95). Ignite slowly in crucible at temp. below  $900^\circ$  (preferably in muffle furnace with pyrometric control) until C is burned, and then at ca  $1100^\circ$  for 1–2 hours. Cool, and weigh.

The residue consists of  $\text{Mg}_2\text{P}_2\text{O}_7$  and possibly  $\text{Mn}_2\text{P}_2\text{O}_7$  and  $\text{Ca}_3(\text{PO}_4)_2$ . If alcoholic filtrate is clear, the  $\text{Ca}_3(\text{PO}_4)_2$  will not exceed 0.3 mg and may be neglected. Correct for Mn as follows: Dissolve residue in 10 ml of  $\text{H}_2\text{SO}_4$  (1+9); transfer soln to 250 ml Erlenmeyer flask; and add 50 ml of  $\text{HNO}_3$  (1+3), 2 ml of sirupy  $\text{H}_3\text{PO}_4$  (sp. gr. 1.7), and 0.2 g of  $\text{KIO}_4$ . Boil 15–20 min., cool, and dilute to convenient volume. In another flask containing same amounts of reagents treated in similar way, match color by adding a standard soln of  $\text{KMnO}_4$ , or compare with a standard soln of  $\text{KMnO}_4$  in colorimeter. From volume of soln of permanganate required, or reading of colorimeter, calculate weight of  $\text{Mn}_2\text{P}_2\text{O}_7$  in residue. Subtract this weight from total weight, and regard difference as  $\text{Mg}_2\text{P}_2\text{O}_7$ , which contains 21.84% of Mg.

53

*Method II<sup>18</sup>—Official, first action*

Weigh 2.5 g of fertilizer into 250 ml volumetric flask, add 30 ml of  $\text{HNO}_3$  and 10 ml of  $\text{HCl}$ , and boil 30 min. Cool, make to volume, and mix. Transfer an aliquot of the clear soln containing not more than 12 mg of Mg to a beaker. Partially neutralize with  $\text{NH}_4\text{OH}$ . Add a few drops of methyl red. Add  $\text{NH}_4\text{OH}$  until soln is yellow, then  $\text{HCl}$  until barely pink. Add 10 ml of saturated soln of  $\text{NH}_4$  oxalate for each 50 ml of soln, adjust soln to pH 5.0 (a faint pink color) by addition of  $\text{HCl}$  (1+4), or  $\text{NH}_4\text{OH}$  (1+4), boil a few minutes, cool, and again adjust reaction to pH 5.0, adding more methyl red if necessary. Stir thoroly and allow soln to stand until precipitate settles. Filter thru a 11 cm paper fine enough to retain Ca oxalate and wash 10 times with hot  $\text{H}_2\text{O}$ . Evaporate filtrate to a volume of ca 100 ml and add 5 ml of a 10% citric acid soln and enough  $\text{NH}_4\text{OH}$  to make soln alkaline to bromothymol blue. Add 5 ml of 10% soln of  $(\text{NH}_4)_2\text{HPO}_4$ . Stir vigorously until precipitation is complete. Add 15 ml of  $\text{NH}_4\text{OH}$  and allow to stand at least 2 hours, stirring frequently, or allow to stand overnight. Transfer precipitate to a small filter or filtering crucible. Wash, and ignite as directed under 52. If  $\text{Mn}_2\text{P}_2\text{O}_7$  is present, correct for it as directed under 52.

54

*Method III. Volumetric Modification—Tentative*

Filter the precipitate of  $\text{MgNH}_4\text{PO}_4$  from 53 thru asbestos pad on Gooch crucible. Remove excess  $\text{NH}_3$  by washing with a soln of equal volumes of alcohol and  $\text{H}_2\text{O}$  (6–10 washings). Transfer pad and precipitate quantitatively to beaker with  $\text{H}_2\text{O}$ . To ca 50 ml add sufficient 0.1 N  $\text{H}_2\text{SO}_4$  from buret to dissolve precipitate, and use a small excess of the acid. Titrate excess acid with 0.1 N  $\text{NaOH}$ , using methyl orange as indicator. 1 ml of 0.1 N acid = 0.00122 g of Mg.

If Mn is present, add 1 ml of  $\text{H}_2\text{SO}_4$  to the soln from above titration, and transfer to 200 ml volumetric flask. Make to volume, mix, and pipet 50 ml of the clear soln into beaker. Add 5 ml of 85%  $\text{H}_3\text{PO}_4$  and 0.3 g of  $\text{KIO}_4$  and heat 30 min., or until color development is complete. Dilute to measured volume containing not more than 20 p.p.m. of Mn and compare with a  $\text{KMnO}_4$  standard in a colorimeter.

Correct the previous titration, or calculated weight of Mg, for the Mn present, taking account of the dilutions.

55 **MAGNESIUM IN WATER-SOLUBLE COMPOUNDS<sup>18</sup>—OFFICIAL, FIRST ACTION**

(Applicable to sulfate of potash magnesia, sulfate of magnesia, and kieserite.)

Weigh 1 g sample into 250 ml volumetric flask, add 200 ml of  $\text{H}_2\text{O}$ , and boil for 30 min.; cool, and dilute to volume with  $\text{H}_2\text{O}$ . Proceed as directed in 53, beginning "Transfer an aliquot of the clear soln."

## ACID-SOLUBLE MANGANESE

56

## REAGENTS

(a) *Potassium permanganate*.—0.0910 *N*. 2.876 g of  $\text{KMnO}_4$  in 1 liter of solution. Standardize with Na oxalate.

(b) *Ferrous sulfate*.—0.0910 *N*. 25.3 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and 25 ml of  $\text{H}_2\text{SO}_4$  in 1 liter of soln. Standardize with the 0.0910 *N*  $\text{KMnO}_4$ .

(c) *Mercuric nitrate*.—10%. Dissolve 10 g of  $\text{Hg}(\text{NO}_3)_2$  in 90 ml of  $\text{H}_2\text{O}$  and clear with a few drops of  $\text{HNO}_3$ .

57

*I. For Manganese Salts and Fertilizers<sup>19</sup>—Official, first action*

Weigh 1 g of sample into beaker, and add 30 ml of  $\text{HNO}_3$  and 10 ml of  $\text{HCl}$ . Cover beaker with cover-glass supported above rim of beaker and digest at boiling point 30 min., adding more of the acids to prevent evaporation to dryness. To the soln, or to aliquot if more than 30 mg of Mn is present, add 15 ml of mixture of acids consisting of 1 part of  $\text{H}_2\text{SO}_4$ , 7 parts of 85%  $\text{H}_3\text{PO}_4$ , and 7 parts of  $\text{H}_2\text{O}$ , by volume, and evaporate to white fumes in the covered beaker. If organic residues remain at this point, add successive portions of 5 ml of  $\text{HNO}_3$ , repeating evaporation after each addition until organic matter is destroyed and chlorides are expelled. Dilute to volume of 50–75 ml with  $\text{H}_2\text{O}$ . Add  $\text{KIO}_4$  at rate of 0.3 g for each 15 mg of Mn. Avoid large excess to save time in filtration later. Heat below boiling point 30 min. Dilute to 150 ml, cool, and add 25 ml of the  $\text{Hg}(\text{NO}_3)_2$ . Stir four times within 3 min. and filter precipitate of I salts on pad of asbestos on Gooch crucible, using suction. Wash with  $\text{H}_2\text{O}$  until washings are no longer pink. Reduce the  $\text{KMnO}_4$  in filtrate with accurately measured volume of 0.0910 *N*  $\text{FeSO}_4$ , using small excess. Titrate excess  $\text{FeSO}_4$  with 0.0910 *N*  $\text{KMnO}_4$ . 1 ml of 0.0910 *N*  $\text{FeSO}_4$  = 1 mg of Mn.

58

*II. For Fertilizers—Tentative*

(Applicable for determinations of low percentages.)

Place 1 g of sample in 200 ml wide-neck volumetric flask or a 250 ml beaker. Add 10 ml of  $\text{H}_2\text{SO}_4$  and 30 ml of  $\text{HNO}_3$ . Heat gently until brown fumes diminish, then boil 30 min. If organic matter is not destroyed, cool, add 5 ml of  $\text{HNO}_3$  and boil. Repeat this process until no organic matter remains, and boil until white fumes appear. Cool slightly, and add 50 ml of  $\text{H}_3\text{PO}_4$  soln (90 ml of  $\text{H}_2\text{O}$ , 10 ml of 85%  $\text{H}_3\text{PO}_4$ ). Boil for a few minutes. Cool, make to 200 ml in a volumetric flask, mix, and let stand to allow precipitation of  $\text{CaSO}_4$ . Pipet 50 ml of clear soln into a beaker. Continue as directed in 57, beginning "Add  $\text{KIO}_4$  at rate of 0.3 g for each 15 mg of Mn, etc." At final dilution soln should contain not more than 20 p.p.m. of Mn. Calculate to Mn.

ACID-FORMING OR NON-ACID-FORMING QUALITY<sup>20</sup>—TENTATIVE

59

## REAGENTS

(a) *Mixed indicator*.—Weigh 0.1 g of bromcresol green and 0.02 g of methyl orange into agate mortar, triturate, and slowly add ca 2 ml of 0.1 *N*  $\text{NaOH}$ . Dilute to 100 ml with  $\text{H}_2\text{O}$ .

(b)  *$\text{Na}_2\text{CO}_3$ -sucrose soln*.—Dissolve 106 g of anhydrous  $\text{Na}_2\text{CO}_3$ , or 286 g of  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ , and 50 g of sucrose, in  $\text{H}_2\text{O}$ . Dilute to 1 liter. Pipet 10 ml into 250 ml Erlenmeyer flask, add 30 ml of normal  $\text{HCl}$  soln carefully, and boil gently few



minutes to remove  $\text{CO}_2$ . Titrate with 0.5 *N* NaOH as directed below. The number of ml of 0.5 *N* NaOH used in titrating is the blank for the soln.

## 60

## DETERMINATION

If the fertilizer mixture, ground as directed under 2, contains less than 30% as the sum of percentages of total N, available  $\text{P}_2\text{O}_5$ , and water-soluble potash, weigh 1 g of mixture into 100 or 150 ml porcelain or Pyrex glass beaker. If the sum of these percentages is 30 or more, use 0.5 g, and for salts of Na or K use 0.25 g. With a pipet or buret add 10 ml of the  $\text{Na}_2\text{CO}_3$ -sucrose soln, and mix thoroly with the fertilizer, except for unmixed nitrate salts. For these, substitute 0.25 g of carbon black for the sucrose. Place in sand bath to depth of mixture in beaker and evaporate to complete dryness. (A cone of ashless filter paper folded so that the base will just slip into the beaker and touch the sides all around, with apex cut off to form a vent ca 3 mm in diameter, may be used to avoid loss by spattering.) Place beakers in furnace heated to ca 250°, and raise temp. gradually to 500–600° (dull red). Hold at this temp. 1 hour. (It is not necessary that all carbon be removed.) Remove beaker and allow to cool. Add 50 ml of  $\text{H}_2\text{O}$ , cover with watch-glass, and add 30 ml of normal HCl thru lip of beaker. After effervescence ceases, place covered beaker on hot plate or steam bath and maintain just below boiling point 1 hour. Filter soln thru disk of paper, or a pad of asbestos that has been digested with normal HCl and washed free from acid with  $\text{H}_2\text{O}$ , using Gooch crucible and suction. Wash with hot  $\text{H}_2\text{O}$ . To clear filtrate (ca 100 ml) add 0.4 ml of the mixed indicator, and titrate to a light green color (pH 4.3) until the green definitely predominates over the yellow. (A duplicate soln of the fertilizer ash displaying maximum acid color for this indicator may be used as comparison to determine first change. Titration is conveniently carried out on white porcelain plate and by using artificial daylight bulb placed at convenient angle above and back of porcelain plate.)

Subtract algebraically ml of 0.5 *N* NaOH used in titrations from the blank, 59(b). For 1 g sample multiply result by 50; 0.5 g sample, by 100; 0.25 g sample, by 200. Positive values represent excess base in ash in pounds of  $\text{CaCO}_3$  equivalent per ton of fertilizer. Negative values represent excess acidity in same terms.

Percentage of N found  $(25 \text{ or } 27) \times 35.7$  is considered the acid-forming power of the N in terms of pounds of  $\text{CaCO}_3$  equivalent per ton of fertilizer, and is given a negative sign in calculating net acid-base balance.

Percentage of citrate-insoluble  $\text{P}_2\text{O}_5$ ,  $(16) \times 28.2$  = alkalinity equivalent to 2 of the 3 Ca atoms of  $\text{Ca}_3(\text{PO}_4)_2$  expressed as pounds of  $\text{CaCO}_3$  equivalent per ton of fertilizer. Correct net balance for fertilizer for this basicity, assumed to be relatively inactive in the soil, by giving the value a negative sign.

The algebraic sum of the acid-base balance of the ash and the corrections for N and citrate-insoluble  $\text{P}_2\text{O}_5$  is the net balance of the fertilizer as pounds of  $\text{CaCO}_3$  equivalent per ton. If negative, the fertilizer is considered acid forming; if positive, it is considered non-acid forming.

## BASIC SLAG

## 61

## MECHANICAL ANALYSIS—OFFICIAL

Proceed as directed under 3, using 10 g of material.

## 62

## PREPARATION OF SAMPLE—OFFICIAL

Proceed as directed under 2.

## TOTAL PHOSPHORIC ACID

*Gravimetric Method—Official*

63

## PREPARATION OF SOLUTION

Proceed as directed under 8(b), or use HCl alone. In latter case, measure out portion for analysis, add 3–5 ml of  $\text{HNO}_3$ , and heat few minutes.

64

## DETERMINATION

Dehydrate aliquot (20 ml) of prepared soln by evaporating to dryness on steam or hot water bath. Treat with 5 ml of HCl and 25 ml of hot  $\text{H}_2\text{O}$ , digest in order to complete the soln, and filter off  $\text{SiO}_2$ . Proceed as directed under 9. Before precipitating with magnesia mixture, add 5 ml of 5% Na acetate soln.

65

*Volumetric Method—Official*

Proceed as directed under 8(b) and determine  $\text{P}_2\text{O}_5$  in aliquot of this soln as directed under 12, standardizing solns against a standard phosphate material of approximately same composition as sample under examination.

CITRIC ACID-SOLUBLE PHOSPHORIC ACID<sup>21</sup>*Gravimetric Method—Official*

66

## PREPARATION OF SOLUTION

Weigh 5 g of prepared slag, 2, into a 500 ml cylindrical shaking flask (Wagner) containing 5 ml of alcohol. (Neck of flask should be at least 22 mm wide, and graduation marks at least 8 cm below mouth.) Make up to mark with 2% citric acid soln at 17.5°. Fit flask with rubber stopper and place at once in rotary apparatus, shaking flask 30 min. at 30–40 r.p.m. Filter immediately on dry filter and analyze soln at once.

67

## DETERMINATION

To 50 ml of the clear filtrate in beaker add 100 ml of molybdate soln, 7(a), and place beaker in water bath; when temp. of contents reaches 65°, remove beaker and cool to room temp. Filter, and wash yellow precipitate of  $\text{NH}_4$  phosphomolybdate 4 or 5 times with 1%  $\text{HNO}_3$ . Dissolve precipitate in 100 ml of cold 2%  $\text{NH}_4\text{OH}$ , and nearly neutralize with HCl. Add to soln dropwise, with continuous stirring, 15 ml of magnesia mixture, 7(c), and proceed as directed under 9.

68

*Volumetric Method—Official*

In an aliquot of the clear soln, 66, determine  $\text{P}_2\text{O}_5$  as directed under 12.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 4, 594 (1921); 5, 315 (1922); 12, 98 (1929).
- <sup>2</sup> Ibid., 15, 66 (1932).
- <sup>3</sup> Ibid., 13, 38, 203 (1930).
- <sup>4</sup> Ibid., 5, 92 (1921); 443 (1922); 6, 384 (1923); 16, 68 (1933); 17, 62 (1934).
- <sup>5</sup> Ibid., 5, 460 (1922); 22, 254 (1939).
- <sup>6</sup> Ibid., 19, 107, 194 (1936); 22, 70 (1939).
- <sup>7</sup> Chem. Ztg., 16, 1952 (1892); J. Ind. Eng. Chem., 11, 306 (1919); 12, 352 (1920); J. Assoc. Official Agr. Chem., 5, 450 (1922); 6, 391 (1923); 15, 66 (1932).
- <sup>8</sup> J. Ind. Eng. Chem., 11, 465 (1919); 21, 77 (1938).

- <sup>9</sup> J. Assoc. Official Agr. Chem., 13, 38 (1930); 20, 67 (1937); 21, 77 (1938).  
<sup>10</sup> Ibid., 10, 198 (1927); 13, 208 (1930).  
<sup>11</sup> Ibid., 18, 62 (1935); 19, 68, 279 (1936); 21, 77 (1938); 22, 70 (1939).  
<sup>12</sup> Ibid., 11, 34 (1928); 13, 39, 215 (1930).  
<sup>13</sup> Ibid., 6, 399 (1923); 7, 382 (1924); 18, 63 (1935); 19, 68, 302 (1936).  
<sup>14</sup> Ibid., 5, 80 (1921); 327 (1922).  
<sup>15</sup> J. Am. Chem. Soc., 20, 288 (1898); J. Assoc. Official Agr. Chem., 5, 88 (1921).  
<sup>16</sup> Leach, Food Inspection and Analysis, 4th ed., 1920, p. 884.  
<sup>17</sup> J. Assoc. Official Agr. Chem., 11, 34 (1928); 16, 69 (1933).  
<sup>18</sup> Ibid., 19, 68 (1936); 20, 252 (1937); 22, 270 (1939).  
<sup>19</sup> Ibid., 21, 292 (1937); 22, 71, 279 (1938).  
<sup>20</sup> Ibid., 18, 236 (1935); 21, 77 (1937); 22, 289 (1939).  
<sup>21</sup> Ibid., 6, 123 (1922); 7, 218 (1924).

---

### III. SEWAGE\*

\*. See note at bottom of p. ix.

## IV. AGRICULTURAL LIMING MATERIALS<sup>1</sup>

1

### DIRECTIONS FOR SAMPLING—TENTATIVE

Take sample representative of lot or shipment and that does not contain disproportionate quantity of the surface or of any modified, or damaged zone, in following manner:

(a) *Burnt, or lump lime, in bulk.*—Collect composite sample of not less than 10 shovelfuls per car, with proportionate quantities from smaller lots, taking each shovelful from different part of lot or shipment. Crush immediately to pass circular opening 1" in diameter, mix thoroly and rapidly, quarter down to 5 lb sample, and place in properly labeled, dry, air-tight container.

(b) *Burnt, or lump lime, in barrels.*—Select at random 5 barrels from each lot or shipment of 20 tons or less and 1 additional barrel for each additional 5 tons. Take not less than 10 lbs from each barrel selected and treat as directed under (a).

(c) *Hydrated lime and ground burnt lime, in bags.*—Select 10 bags from different parts of each lot or shipment of 20 tons or less and 1 additional bag for each additional 5 tons. From each of bags sampled withdraw core from top to bottom by means of sampling tube, mix these portions thoroly and rapidly on heavy sized paper or oilcloth, quarter down to 2 lb sample, and place in properly labeled, dry, air-tight container.

(d) *Ground limestone and ground marl, in bags.*—Proceed as directed under (c).

(e) *Ground limestone, ground burnt lime, and ground marl, in bulk.*—By means of slotted sampling tube, withdraw samples to full sampler depth from 10 points in lot or shipment, and proceed as directed under (c), beginning "mix these portions."

2

### PREPARATION OF SAMPLE—TENTATIVE

Grind sample in porcelain mortar or porcelain ball mill to pass 60-mesh sieve, mix thoroly, and preserve in air-tight container.

### NEUTRALIZING VALUE—TENTATIVE

3

#### REAGENTS

(a) *Sodium hydroxide soln.*—0.25 *N*. Prepare free from carbonates and store in bottle provided with siphon tube and with guard tubes containing soda-lime, or other suitable device, to prevent absorption of CO<sub>2</sub> from air.

(b) *Nitric acid.*—0.5 *N*. Standardize against (a), using phenolphthalein indicator, II, 10(d).

4

#### DETERMINATION

Place 0.5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 2, in 150 ml beaker; add 50 ml of the HNO<sub>3</sub>, cover beaker with watch-glass, and boil 5 min. Cool, and titrate excess of acid with the NaOH soln, using phenolphthalein indicator. Report as percentage of CaO in burnt and hydrated lime and as percentage of CaCO<sub>3</sub> equivalent for limestone and marl.

### CAUSTIC VALUE<sup>2</sup>—OFFICIAL

5

#### APPARATUS

In illustration (Fig. 6), *A* is 500 ml Erlenmeyer flask of Pyrex glass, and *F* is filter cone packed nearly full with cotton, which is covered to depth of 2–3 mm

with lightly compacted, macerated filter paper. Filter cone is connected with syphon tube *B* by means of thick-walled rubber tubing. Receiving flasks *m* and *n* are calibrated to deliver 50 and 100 ml, respectively. *S* is suction flask.

6

## DETERMINATION

Transfer portion of sample, *2*, to weighing bottle and determine weight of bottle and contents in an atmosphere of minimum moisture and CO<sub>2</sub> content. By means of polished, narrow-pointed spatula calibrated to hold ca 1.5 g, withdraw charge to be used and determine its exact weight by difference. Introduce charge directly into dry flask (*A*), provided with tightly fitting rubber stopper.

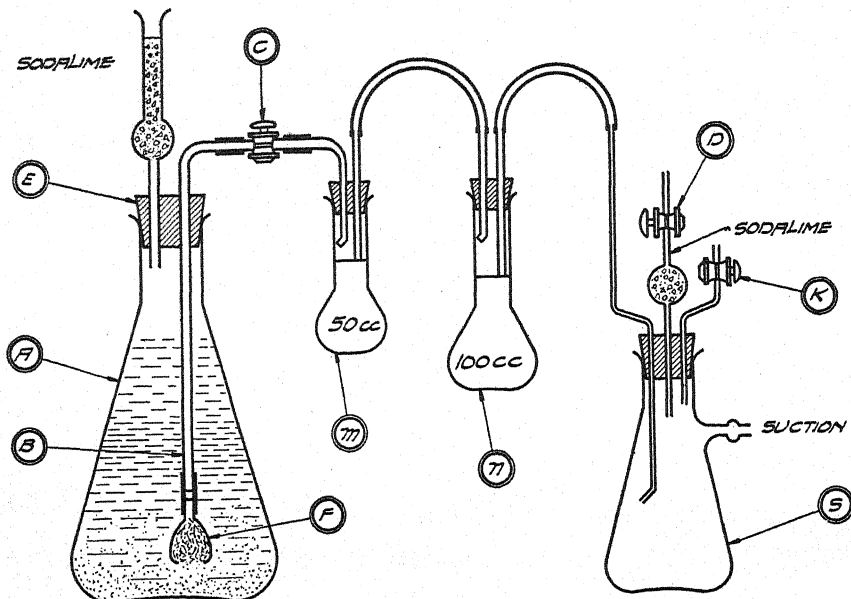


FIG. 6.—APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS

Prepare a sugar soln *immediately before use* by placing 25 g of granulated sugar in measuring flask calibrated to deliver 500 ml. Dissolve sugar with cold CO<sub>2</sub>-free H<sub>2</sub>O and make to mark. Holding both Erlenmeyer flask containing charge and flask containing sugar soln in a slightly inclined position, insert neck of sugar soln flask short distance into Erlenmeyer flask, and carefully transfer sugar soln while simultaneously and synchronously agitating both flasks by rotary motion to prevent granulation of lime. Stopper Erlenmeyer flask securely; agitate; and add, if desired, quantity of clean dry beads. Effect complete soln of uncoated caustic lime by six 1-min. agitations at intervals of 2 or 3 min. Crush any undisintegrated particles of sample by careful twisting of stopper after inverting flask to trap them in space between stopper and neck of flask. Allow 15 min. further contact between lime and sugar soln, and filter.

Connect filter cone *F* with syphon *B* and close stopcock *D*. Connect receiving flasks, apply suction, and quickly connect Erlenmeyer flask (*A*) containing lime soln with stopper *E*. Open stopcock *C* and filter 25–50 ml of soln. Close *C* and open *D* to release suction. Remove *m* and replace with another dry flask of same kind.

Close *D*, open *C*, and continue filtration until both *m* and *n* have been filled at least to the marks. To disconnect system, close stopcock *C* and press outlet of flask *m* down gently and then outlet of flask *n* to remove any excess of liquid above the marks. Permit intermediate connection to empty, open stopcock *D*, and remove *m* and *n*. Titrate first 50 ml, or pilot aliquot, of filtered soln with 0.5 *N* HCl, using phenolphthalein indicator. Run twice the volume of 0.5 *N* acid required for this titration into covered 200 ml beaker; add second, or 100 ml aliquot, of filtered soln to this acid and phenolphthalein indicator; and complete titration.

Calculate caustic value of sample by the formula:

$$X = \frac{7A}{W}, \text{ in which}$$

*X* = percentage of active CaO;

*A* = ml of 0.5 *N* acid used per 100 ml of lime soln;

*W* = weight of charge.

7

#### CARBON DIOXIDE—TENTATIVE

Proceed as directed under I, 6, using 5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 2. Report as percentage of CaCO<sub>3</sub>.

8

#### TOTAL CALCIUM OXIDE—TENTATIVE

Place 1 g of burnt or hydrated lime (2 g of ground limestone or ground marl), prepared as directed under 2, in hard glass beaker of 250 ml capacity; add 25 ml of H<sub>2</sub>O, 10 ml of HCl, and few drops of HNO<sub>3</sub>; boil 10 min.; and evaporate to dryness. Separate and remove insoluble matter, SiO<sub>2</sub>, and Fe and Al oxides, as directed under I, 12 and 13. Determine CaO as directed under I, 14.

9

#### TOTAL MAGNESIUM OXIDE—TENTATIVE

Proceed as directed under I, 16, using combined filtrate and washings from CaO determination, 8.

10

#### MECHANICAL ANALYSIS OF GROUND LIMESTONE—TENTATIVE

Transfer 100 g of original material to set of 10-, 20-, 40-, 60-, 80-, and 100-mesh standardized sieves that comply with specifications of Bureau of Standards. Sift, shaking 5 min. on the 80- and 100-mesh sieves and breaking lumps by means of a soft rubber pestle if the material has a tendency to cake. Weigh material retained on each sieve and that passing 100-mesh sieve and report as percentages of total weight.

#### SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 7, 252 (1924); U. S. Bur. Standards Circs., 96, 118, 143, 150, 153; Am. Soc. Test. Materials, Tentative Standards, 1923, p. 277.  
<sup>2</sup> Ind. Eng. Chem., 20, 312 (1928); J. Assoc. Official Agr. Chem., 11, 153 (1928); 14, 283 (1931).

---

#### V. AGRICULTURAL DUST\*

\* See note at bottom of p. ix.

## VI. INSECTICIDES AND FUNGICIDES

### GENERAL METHODS

1

#### PREPARATION OF SAMPLE—OFFICIAL

Thoroughly mix all samples before analysis. Make water-soluble As determinations on samples as received, without further pulverization or drying. In the case of lye, NaCN, or KCN, weigh large quantities in weighing bottles and analyze aliquots of the aqueous solns.

2

#### MOISTURE—OFFICIAL

(Applicable to Paris green, London purple, powdered lead arsenate, calcium arsenate, magnesium arsenate, zinc arsenite, and powdered Bordeaux mixture.)

Dry 2 g to constant weight at 105–110° and report loss in weight as moisture.

#### TOTAL ARSENIC

##### *I. By Cuprous Chloride Distillation<sup>1</sup>—Official*

(Applicable except in presence of nitrates to determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

3

#### REAGENTS

(a) *Standard arsenious oxide soln.*—Dissolve 2 g of pure  $\text{As}_2\text{O}_3$  in beaker by boiling with 150–200 ml of  $\text{H}_2\text{O}$  containing 10 ml of  $\text{H}_2\text{SO}_4$ , cool, transfer to 500 ml volumetric flask, and dilute to mark.

(b) *Standard iodine soln.*—Approximately 0.05 *N*. Mix 6.35 g of pure I with twice this weight of pure KI, dissolve in small quantity of  $\text{H}_2\text{O}$ , filter, and dilute filtrate to 1 liter in volumetric flask. Standardize against (a) as follows: Pipet 50 ml of the  $\text{As}_2\text{O}_3$  soln into Erlenmeyer flask, dilute to same volume as that of aliquot used for titration in actual determination, neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, and add the standard I soln from a buret, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml of the starch indicator (e) and continue adding the I soln, dropwise, until a permanent blue color is obtained. Calculate value of the standard I soln in terms of  $\text{As}_2\text{O}_3$  and  $\text{As}_2\text{O}_5$ . For conversion of  $\text{As}_2\text{O}_3$  to  $\text{As}_2\text{O}_5$  multiply by 1.1617. Occasionally restandardize the I against the standard  $\text{As}_2\text{O}_3$  soln.

(c) *Standard bromate soln.*—Dissolve 1.525 g of  $\text{NaBrO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter; 1 ml of this soln is approximately equal to 0.003 g of  $\text{As}_2\text{O}_3$ . Standardize against (a) as follows: Pipet 25 ml aliquots of the  $\text{As}_2\text{O}_3$  soln into 500 ml Erlenmeyer flasks, add 15 ml of HCl, dilute to 100 ml, heat to 90°, and titrate with the bromate soln, using 10 drops of the methyl orange indicator (f). Do not add indicator until near end of titration, and agitate liquid continuously in order to avoid local excess of the bromate soln. Add bromate soln very slowly when approaching end of titration; end point is shown by change from red to colorless.

(d) *Sodium hydroxide soln.*—Dissolve 400 g of NaOH in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(e) *Starch indicator.*—Mix ca 2 g of finely powdered potato starch with cold  $\text{H}_2\text{O}$  to thin paste; add ca 200 ml of boiling  $\text{H}_2\text{O}$ , stirring constantly, and immediately discontinue heating. Add ca 1 ml of Hg, shake, and allow the starch to stand over the Hg.

(f) *Methyl orange indicator*.—Dissolve 0.5 g of methyl orange in  $\text{H}_2\text{O}$  and dilute to 1 liter.

4

## APPARATUS

Fig. 7.—Distillation flask is of 500 ml capacity and rests on metal gauze that fits over circular hole in heavy sheet of asbestos board, which in turn extends out far enough to protect sides of flask from direct flame of burner. First receiving flask holds 500 ml and contains 40 ml of  $\text{H}_2\text{O}$ ; second holds 500 ml and contains 100 ml of  $\text{H}_2\text{O}$ . Volume in first flask should not exceed 40 ml, otherwise there may be

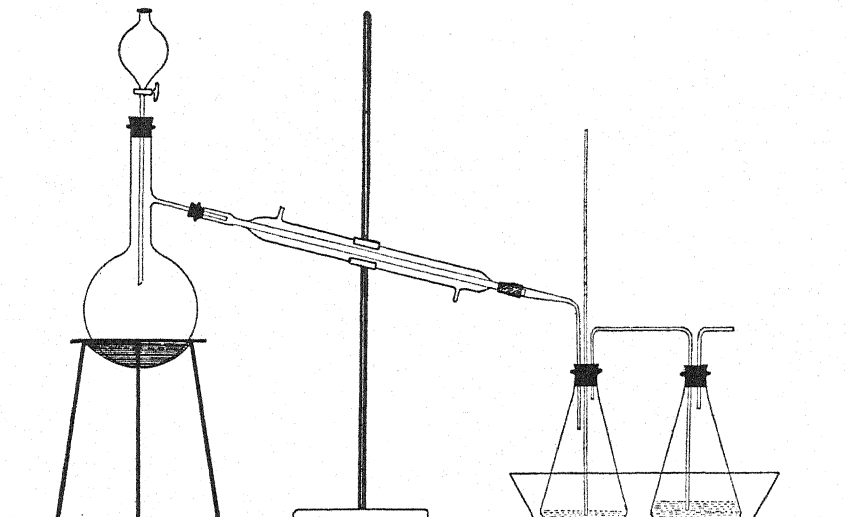


FIG. 7.—APPARATUS FOR DISTILLATION OF ARSENIOS CHLORIDE

separated a compound of As that can not readily be redissolved without danger of loss of  $\text{AsCl}_3$ . Keep both flasks cool by placing them in pan thru which  $\text{H}_2\text{O}$  circulates, or which contains  $\text{H}_2\text{O}$  and pieces of ice.

5

## DETERMINATION

Weigh quantity of sample containing not more than 0.4 g of As and wash into distillation flask by means of 100 ml of  $\text{HCl}$ . Add 5 g of  $\text{Cu}_2\text{Cl}_2$  and distil. When volume in distillation flask is reduced to ca 40 ml add 50 ml more of  $\text{HCl}$  by means of dropping funnel and continue distillation, repeating addition of 50 ml portions of  $\text{HCl}$  until 200 ml of the acid distillate has passed over. Wash down condenser and all connecting tubes carefully, transfer these washings and contents of Erlenmeyer flasks to liter volumetric flask, dilute to mark, and mix thoroly. Titrate distillate by one of following procedures:

(a) Pipet 200 ml aliquot into Erlenmeyer flask and nearly neutralize with the  $\text{NaOH}$  soln, using few drops of phenolphthalein indicator, II, 10(d), and keeping soln well cooled. If neutral point is passed, add  $\text{HCl}$  until again slightly acid. Neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, and add the standard I soln from buret, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml of the starch indicator and continue adding the I soln dropwise until permanent blue color is obtained.



(b)<sup>2</sup> Pipet 200 ml aliquot into Erlenmeyer flask and titrate with the bromate soln, 3(c), beginning "heat to 90°."

From number of ml of standard soln used, calculate percentage of As in sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$ , according to whether the As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

## II. By Hydrazine Sulfate Distillation<sup>3</sup>—Official

(Nitrates do not interfere in this method. Applicable to determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

6

### REAGENTS

*Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$  and 20 g of NaBr in 1 liter of HCl (1+4). See 3 for other reagents and solns.

7

### APPARATUS.—See 4.

8

### DETERMINATION

Weigh quantity of sample containing not more than 0.4 g of As and transfer to distilling flask. Add 50 ml of the  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$ -NaBr soln, close flask with the stopper that carries funnel tube, and connect side tube with condenser. Boil 2–3 min., add 100 ml of HCl by means of the dropping funnel, and distil until volume in distilling flask is reduced to ca 40 ml; add 50 ml more of HCl and continue distillation until contents of flask are again reduced to ca 40 ml. Wash down condenser, transfer contents of receiving flask to a liter volumetric flask, dilute to volume, and mix thoroly. Titrate distillate—

(a) As directed under 5(a); or

(b) Pipet a 200 ml aliquot into an Erlenmeyer flask, add 10 ml of HCl, and titrate with the standard bromate soln, 3(c), beginning "heat to 90°."

From number of ml of standard soln used, calculate percentage of As in sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$ , according to whether the As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

## Method III<sup>4</sup>—Tentative

(Applicable in presence of sulfides, sulfites, thiosulfates, or large quantities of sulfur.)

9

### REAGENTS

*Sodium thiosulfate soln.*—Approximately 0.05 N. Dissolve 31 g of crystallized  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. See 3 for other reagents and solns and 4 for the apparatus.

10

### DETERMINATION

Weigh 2 g of sample and transfer to distilling flask. Add soln of 5–8 g of  $\text{Cu}_2\text{Cl}_2$  in 100 ml of HCl and shake to bring sample completely in contact with the acid soln and to expel  $\text{H}_2\text{S}$ . When reaction has ceased, close flask, connect with condenser, and distil as directed under 5 until 200 ml of acid distillate has passed over. Make distillate to volume in liter flask, mix thoroly, and transfer 200 ml aliquot to 400 ml Pyrex beaker or porcelain casserole. Add 10 ml of  $\text{HNO}_3$  and 5 ml of  $\text{H}_2\text{SO}_4$ , evaporate to sirupy consistency on steam bath, then heat on hot plate until white fumes of  $\text{H}_2\text{SO}_4$  appear. Cool, and wash into 500 ml Erlenmeyer flask. If quantity of  $\text{H}_2\text{SO}_4$  is appreciably lessened by fuming, add sufficient to make total

quantity of  $\text{H}_2\text{SO}_4$  ca 5 ml. Dilute to 100–150 ml, add 1.5 g of KI, and boil until volume is reduced to ca 40 ml. Cool soln under running  $\text{H}_2\text{O}$ , dilute to 100–150 ml, and add the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 9, dropwise until the I color is just removed. Nearly neutralize the  $\text{H}_2\text{SO}_4$  with the NaOH soln, 3(d), finish neutralization with  $\text{NaHCO}_3$ , add 4–5 g in excess, and titrate with the standard I soln as directed under 3(b). From number of ml of standard soln used calculate percentage of As in sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$  according to whether As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

#### WATER-SOLUBLE ARSENIC—OFFICIAL

(Applicable to determination of water-soluble arsenic in lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

11

REAGENTS.—See 3 and 9.

12

#### DETERMINATION

To 2 g of original sample if a powder, or 4 g if a paste, in liter Florence flask, add 1 liter of recently boiled  $\text{H}_2\text{O}$  that has been cooled to  $32^\circ$ . Stopper flask and place in water bath kept at  $32^\circ$  by means of thermostat. Digest 24 hours, shaking hourly for 8 hours during this period. Filter thru dry filter. (If filtrate is not clear, refilter thru Büchner funnel containing paper and sufficient coating of filter-cel to give a clear soln. Discard first 50 ml.) Transfer 250–500 ml of *clear* filtrate to Erlenmeyer flask, add 3 ml of  $\text{H}_2\text{SO}_4$ , and evaporate on hot plate. When volume reaches ca 100 ml add 1 g of KI, and continue boiling until volume is ca 40 ml. Cool, dilute to ca 200 ml, and add the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 9, dropwise, until I color is exactly removed. (Avoid use of starch indicator at this point.) Neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, titrate with the standard I soln until yellow color disappears slowly, add 5 ml of the starch indicator, and continue titration to permanent blue color. Make correction for quantity of standard I soln necessary to produce the same color, using same reagents and volume. From number of ml of standard I soln used calculate percentage of water-soluble As in sample.

13

#### LEAD OXIDE\*—OFFICIAL\*

Weigh 1 g of powdered sample and transfer to a beaker. Add 5 ml of HBr (ca 1.38 sp. gr.) and 15 ml of HCl, and evaporate to dryness to remove As. Repeat treatment, add 20 ml more of the HCl, and again evaporate to dryness. Add to residue 25 ml of 2 N HCl, heat to boiling, filter immediately to remove  $\text{SiO}_2$ , and wash with boiling  $\text{H}_2\text{O}$  to volume of 125 ml. See that all  $\text{PbCl}_2$  is in soln before filtering; if it will not dissolve completely in 25 ml of 2 N acid, add 25 ml additional, and dilute filtrate to 250 ml volume. Pass in  $\text{H}_2\text{S}$  until precipitation is complete. Filter, and wash precipitate thoroly with 0.5 N HCl saturated with  $\text{H}_2\text{S}$ . Save filtrate and washings for determination of Zn. Transfer filter paper containing the sulfides of Pb and Cu to 400 ml Pyrex beaker and completely oxidize all organic matter by heating on steam bath with 4 ml of  $\text{H}_2\text{SO}_4$  and ca 20 ml of fuming  $\text{HNO}_3$  in covered beaker. Evaporate on steam bath and then completely remove  $\text{HNO}_3$  by heating on hot plate until copious evolution of white fumes of  $\text{H}_2\text{SO}_4$  occurs. Cool, add 2–3 ml of  $\text{H}_2\text{O}$ , and again heat to fuming. Cool, add 50 ml of  $\text{H}_2\text{O}$  and 100 ml of alcohol, and let stand several hours (preferably overnight). Filter thru Gooch crucible, previously washed with  $\text{H}_2\text{O}$ , with acidified alcohol (100 parts of  $\text{H}_2\text{O}$ , 200 parts of alcohol, and 3 parts of  $\text{H}_2\text{SO}_4$ ) and with alcohol, and then

\* Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate.

dried at 200°. Wash the precipitate of  $\text{PbSO}_4$  in crucible about 10 times with the acidified alcohol, and then with alcohol, to remove  $\text{H}_2\text{SO}_4$ . Dry at 200° to constant weight, keeping crucible covered to prevent loss from spattering. From weight of  $\text{PbSO}_4$ , calculate percentage of PbO in sample, using factor 0.7360.

#### COPPER\*\*

14

##### *Electrolytic Method—Official*

Evaporate filtrate and washings from the  $\text{PbSO}_4$  precipitation, 13, to fuming; add a few ml of fuming  $\text{HNO}_3$  to destroy organic matter; and continue evaporation until ca 3 ml remains. Take up with ca 100 ml of  $\text{H}_2\text{O}$ , add 1 ml of  $\text{HNO}_3$ , and filter, if necessary. Wash into weighed 150 ml Pt dish and electrolyze, using rotating anode and current of ca 3 amperes. (In lieu of Pt dish a 150 ml beaker and weighed gauze cathode may be used.) After all Cu has been deposited (ca 30 min.) and while current is still flowing, wash deposit with  $\text{H}_2\text{O}$  by siphoning. Interrupt current, rinse cathode with alcohol, dry few moments in oven, and weigh. Calculate percentage of Cu in sample.

15

##### *Thiosulfate Volumetric Method—Official*

Proceed as directed under 14 to point at which filtrate and washings from the  $\text{PbSO}_4$  precipitation have been treated with fuming  $\text{HNO}_3$  and evaporated to volume of ca 3 ml. Take up in ca 50 ml of  $\text{H}_2\text{O}$ , add  $\text{NH}_4\text{OH}$  in excess, and boil until excess  $\text{NH}_3$  is expelled, as shown by change of color in liquid and partial precipitation. Add 3–4 ml of 80% acetic acid, boil 1–2 min., cool, add 10 ml of a 30% KI soln and titrate with standard thiosulfate soln (XXXIV, 40) until the brown color becomes faint. Add starch indicator, 3(e), and continue titration cautiously until blue color due to free I has entirely vanished. From number of ml of standard thiosulfate soln used calculate percentage of Cu in sample.

#### ZINC OXIDE\*—OFFICIAL\*

16

##### REAGENT

*Mercury-thiocyanate soln.*—Dissolve 27 g of  $\text{HgCl}_2$  and 30 g of  $\text{NH}_4\text{SCN}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

17

##### DETERMINATION

Concentrate filtrate and washings from sulfide precipitation, 13, by gentle boiling to ca 50 ml, and continue evaporation on steam bath to dryness. Dissolve residue in 100 ml of  $\text{H}_2\text{O}$  containing 5 ml of  $\text{HCl}$ , and add 35–40 ml of the Hg-thiocyanate reagent with vigorous stirring. Allow to stand at least an hour with occasional stirring. Filter thru a weighed Gooch crucible, wash with  $\text{H}_2\text{O}$  containing 20 ml of the Hg-thiocyanate reagent per liter, and dry to constant weight at 105°. From this weight calculate percentage of  $\text{ZnO}$ , using factor 0.16332.

Some Fe is usually present and during Zn determination should be in ferrous condition. In making the sulfide precipitation the  $\text{H}_2\text{S}$  should be passed into the soln for sufficient time to reduce the Fe, as well as to precipitate the Cu and Pb. The  $\text{ZnHg}(\text{SCN})_4$  precipitate normally is white, and the occluded ferric thiocyanate should not give it more than faint pink color.

\* Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate.

## TOTAL FLUORINE

*I. Lead Chlorofluoride Method<sup>7</sup>—Official, first action*

18

## REAGENTS

(a) *Fusion mixture*.—Mix anhydrous  $\text{Na}_2\text{CO}_3$  and  $\text{K}_2\text{CO}_3$  in equimolecular proportions.

(b) *Lead chlorofluoride wash soln.*—Dissolve 10 g of  $\text{Pb}(\text{NO}_3)_2$  in 200 ml of  $\text{H}_2\text{O}$ ; dissolve 1 g of  $\text{NaF}$  in 100 ml of  $\text{H}_2\text{O}$  and add 2 ml of  $\text{HCl}$ ; mix these 2 solns. Allow precipitate to settle and decant supernatant liquid. Wash 4 or 5 times with 200 ml of  $\text{H}_2\text{O}$  by decantation, and then add ca 1 liter of cold  $\text{H}_2\text{O}$  to the precipitate and allow to stand 1 hour or longer, with occasional stirring. Pour thru filter and use clear filtrate. By adding more  $\text{H}_2\text{O}$  to the precipitate of  $\text{PbClF}$  and stirring, more wash soln may be prepared as needed.

(c) *Standard silver nitrate soln.*—0.2 *N*. Standardize by titration against pure  $\text{NaCl}$ , using  $\text{K}_2\text{CrO}_4$  indicator.

(d) *Standard potassium or ammonium thiocyanate soln.*—0.1 *N*. Standardize by comparing with the standard soln of  $\text{AgNO}_3$  under the same conditions as obtain in the determination.

(e) *Ferric indicator*.—Add to cold saturated soln of ferric alum (free from  $\text{Cl}$ ) sufficient colorless  $\text{HNO}_3$  to bleach the brown color.

(f) *Bromophenol blue indicator*.—Grind 0.1 g of the powder with 1.5 ml of 0.1 *N*  $\text{NaOH}$  soln and dilute to 25 ml.

19

## DETERMINATION

Mix 0.5 g (or less if necessary to make content of F fall between 0.01 and 0.1 g) of sample with 6 g of fusion mixture and 0.2–0.3 g of powdered silica and heat to fusion over Bunsen burner. (Use of blast lamp is not required as it is only necessary that the mass be fluid, and it is preferable not to heat much beyond temp. at which it melts. If much  $\text{Al}$  is present, a uniform, clear, liquid melt cannot be obtained. There will be particles of a white solid separated in the liquid. The melt after cooling should be colorless, or at least should not have more than a gray color.) Leach cooled melt with hot  $\text{H}_2\text{O}$ , and filter when disintegration is complete. Return the insoluble residue to a  $\text{Pt}$  dish by the use of jet of  $\text{H}_2\text{O}$ , add 1 g of  $\text{Na}_2\text{CO}_3$ , make the volume 30–50 ml, boil a few minutes, disintegrating any lumps with glass rod flattened on end, filter thru same paper, wash thoroly with hot  $\text{H}_2\text{O}$ , and adjust volume of filtrate and washings to ca 200 ml. Add 1 g of  $\text{ZnO}$  dissolved in 20 ml of  $\text{HNO}_3$  (1+9), boil 2 min. with constant stirring, filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Return the gelatinous mass to the beaker once or twice and thoroly disintegrate in the wash soln because it is difficult to wash this precipitate on filter. (The mass can easily be returned to beaker by rotating funnel above beaker and at the same time cutting precipitate loose from paper with jet of wash soln.)

Add 2 drops of bromophenol blue and then the  $\text{HNO}_3$  nearly to neutrality, leaving soln slightly alkaline. Boil soln gently with cover-glasses on the beakers, to expel  $\text{CO}_2$ . Finally add  $\text{HNO}_3$  (1+4) until color just changes to yellow. Remove from burners, add dilute  $\text{NaOH}$  until the color just changes to blue, and add 3 ml of 10%  $\text{NaCl}$  soln. Volume of soln at this point should be 250 ml.

Add 2 ml of  $\text{HCl}$  (1+1) and 5 g of  $\text{Pb}(\text{NO}_3)_2$ , and heat on steam bath. As soon as the  $\text{Pb}(\text{NO}_3)_2$  is in soln, add 5 g of  $\text{Na}$  acetate, stir vigorously, and digest on steam bath 30 min. with occasional stirring. Allow to stand overnight at room temp. (4 hours will be sufficient unless much  $\text{B}$  is present.) Decant soln thru a paper of close texture; wash precipitate, beaker, and paper once with cold  $\text{H}_2\text{O}$ ,

then 4 or 5 times with a cool saturated soln of  $\text{PbClF}$  and then once more with cold  $\text{H}_2\text{O}$ .

Transfer precipitate and paper to beaker in which precipitation was made, stir paper to a pulp, add 100 ml of  $\text{HNO}_3$  (5+95), and heat on steam bath until precipitate is dissolved. (Five min. is ample to dissolve this precipitate. If sample contains an appreciable quantity of sulfates the precipitate will contain  $\text{PbSO}_4$ , which will not dissolve. In such a case heat 5–10 min. with stirring and consider the  $\text{PbClF}$  to be dissolved.) Add a slight excess of 0.2 *N*  $\text{AgNO}_3$  soln, digest on steam bath 30 min., cool to room temp. while protected from light, filter, wash with cold  $\text{H}_2\text{O}$ , and determine  $\text{AgNO}_3$  in the filtrate by titration with the standard thiocyanate soln, using 5 ml of the ferric indicator. Subtract quantity of  $\text{AgNO}_3$  found in the filtrate from that originally added. The difference will be that required to combine with the Cl in the  $\text{PbClF}$ , and from this difference calculate percentage of F in sample on basis that 1 ml of 0.2 *N*  $\text{AgNO}_3$  = 0.0038 g of F.

NOTE: This method gives accurate results for quantities of F between 0.01 and 0.10 g. Below 0.01 g the results have a tendency to be slightly low and above 0.1 slightly high. Satisfactory results are obtained in the presence of B and Al. This method should be used for all samples of fluorides that contain kaolin or fullers' earth as a filler.

If sample contains appreciable quantity of S, the S should be removed with  $\text{CS}_2$  and F determined on air-dry residue, allowance being made in calculations for percentage of S removed.

With water-soluble samples, in absence of organic matter or other interfering substances, fusion may be omitted and determination made on aliquot of a water-soluble soln as directed above, beginning "Add 2 drops of bromophenol blue."

### II. Travers' Method (Modified)<sup>8</sup>—Tentative

(Applicable in absence of B, Al, and large quantities of pyrethrum powder.)

#### 20

##### REAGENTS

(a) *Alcoholic potassium chloride soln.*—Dissolve 60 g of KCl in 400 ml of  $\text{H}_2\text{O}$ , add 400 ml of alcohol, and test with phenolphthalein; if soln is not neutral, adjust to exact neutrality by addition of NaOH or HCl.

(b) *Standard sodium hydroxide soln.*—Approximately 0.2 *N*. Prepare in a manner to assure absence of carbonate.

#### 21

##### DETERMINATION

Treat 0.5 g of sample in small beaker with 20–25 ml of  $\text{H}_2\text{O}$ . Add 0.3 g of finely divided precipitated silica and a few drops of methyl orange indicator. Add HCl dropwise until soln assumes an apparently permanent pink color, after which add 2 ml in excess, cover beaker with watch-glass, and boil 1 min. Cool to room temp., add 4 g of solid KCl, and stir until the latter dissolves. Add 25 ml of 95% ethyl alcohol and let stand 1 hour with frequent stirring. Filter thru Gooch crucible containing disk of filter paper covered by medium pad of asbestos. Wash precipitate with the alcoholic KCl soln until one washing does not destroy the color made by 1 drop of 0.2 *N* NaOH soln and phenolphthalein (3–4 washings are usually sufficient). Transfer crucible and contents to 400 ml beaker, add 100 ml of recently boiled  $\text{H}_2\text{O}$  and 1–2 ml of 1% phenolphthalein soln, heat, and titrate with the standard NaOH soln. Finish titration with the fluoride soln actively boiling. Calculate percentage of F present on the basis that 1 ml of 0.2 *N* NaOH soln = 0.0057 g of F.

### III. Distillation Method<sup>9</sup>—Tentative

(Applicable to water-soluble or water-insoluble insecticides in absence of gelatinous  $\text{SiO}_2$ , B, and Al.)

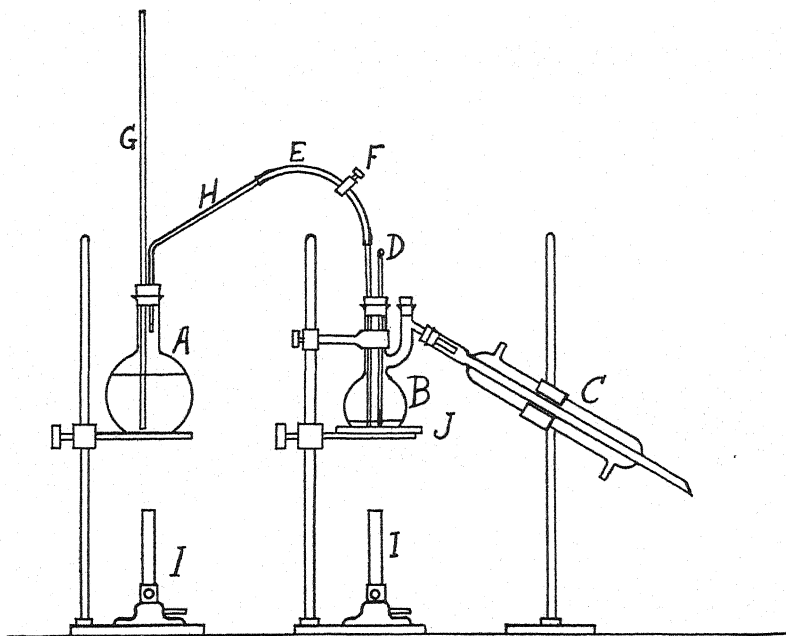


FIG. 8.—APPARATUS FOR DETERMINATION OF FLUORINE

## 22

## REAGENTS

(a) *Sodium alizarin sulfonate indicator*.—Dissolve 0.1 g of sodium alizarin sulfonate in 200 ml of  $H_2O$ .

(b) *Hydrochloric acid* (1+249).—1 part C. P.  $HCl$ , ca 35.4%, to 249 parts of  $H_2O$ .

(c) *Thorium nitrate*.—Approximately 0.05 *N*. In standardizing for use with Procedure (b) add 5 ml of saturated  $KMnO_4$  with the standard  $NaF$ ,  $H_2O$ , and  $H_2SO_4$ .

## 23

## DETERMINATION

(a) *In absence of organic matter*.—Transfer a quantity of sample sufficient to make *F* content ca 0.09 g, with aid of a little  $H_2O$ , to a 250 ml Claissen distillation flask containing 12 glass beads. Adjust volume to ca 30 ml, close flask with a two-holed rubber stopper, thru which passes a thermometer and a 4 mm glass tube, both of which extend down into soln. (The 4 mm glass tube extends ca 1.5" above rubber stopper and by means of rubber tube *E* connects still with 1 liter Florence flask containing  $H_2O$ , which is heated to generate steam. The flask is equipped with a discharge, *H*, a glass tube to release steam, and *G*, a pressure tube.

Bring  $H_2O$  in steam generating flask to b.p. with the pinchcock, *F*, in the release tube open. Connect distilling flask to condenser, add 25 ml of  $H_2SO_4$  thru top of the 4 mm tube by means of pipet or special funnel. With the stopcock, *F*, open, connect the rubber tubing to the 4 mm tube. Light burner under Claissen flask. Regulate flow of steam by adjusting burner flames and stopcock, *F*, so that volume of soln is held constant and a temp. of 145–150° is maintained in flask *B*. Continue

distillation until 400 ml of distillate is collected. Make to volume in 500 ml graduated flask, take one aliquot of 50 ml in a tall-form 150 ml beaker, and add 5 drops of indicator. Adjust acidity with 1% NaOH and HCl (1+250) until pink color is just discharged. Add 2 ml of the HCl soln, and using a buret graduated in 0.05 ml titrate with 0.05 N  $\text{Th}(\text{NO}_3)_4$  to a permanent pink.

Standardize the  $\text{Th}(\text{NO}_3)_4$  in terms of g of F per ml by titrating the F obtained by distillation from NaF of known F content, using the procedure given above.

(b) *In presence of organic matter.*—Transfer a quantity of sample sufficient to make F content ca 0.09 g, with the aid of a little  $\text{H}_2\text{O}$ , to a 250 ml Claisen distillation flask containing 12 beads. Add 5 ml of saturated  $\text{KMnO}_4$  soln, adjust volume to ca 30 ml, and proceed as directed in (a), beginning "close flask with a two-holed rubber stopper."

NOTE: Should a coating of precipitated silica form on the inside of the distillation flask, remove by treatment with hot concentrated alkali soln, as it is capable of retaining F during the distillation of some samples and giving it up, at least in part, in subsequent distillations.

### PARIS GREEN

24

MOISTURE—OFFICIAL.—See 2.

25

TOTAL ARSENIC—OFFICIAL.—See 5 or 8.

#### TOTAL ARSENIOS OXIDE

(Following methods determine only the As present in trivalent form ( $\text{As}_2\text{O}_3$ ). They also determine any Sb that may be present in trivalent form ( $\text{Sb}_2\text{O}_3$ ). Ferrous and cuprous salts vitiate the results.)

#### *Method I<sup>10</sup>—Official*

26

#### REAGENTS

*Ammonium chloride soln.*—Dissolve 250 g of  $\text{NH}_4\text{Cl}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. For other reagents and solns see 3.

27

#### DETERMINATION

Weigh 0.3 g of sample and wash into Erlenmeyer flask with 10–15 ml of HCl (1+4) or 10–15 ml of  $\text{H}_2\text{SO}_4$  (1+4), followed by ca 100 ml of  $\text{H}_2\text{O}$ , and heat on steam bath only long enough to complete soln, at temp. not exceeding  $90^\circ$ . (If  $\text{H}_2\text{SO}_4$  is used soln may be heated to boiling.) Cool, neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, and then add sufficient  $\text{NH}_4\text{Cl}$  soln to dissolve the precipitated Cu. Dilute somewhat and titrate as directed under 3(b). Make correction for quantity of I soln necessary to produce blue color with starch in presence of Cu (using an equivalent weight of  $\text{CuSO}_4$ ). From corrected number of ml standard I soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

28

#### *Method II<sup>11</sup>—Official*

Weigh 1.5 g of sample and wash into 250 ml volumetric flask with 100 ml of HCl (1+4), heating to maximum of  $90^\circ$ , if necessary, to secure complete soln of sample. Cool, and make to volume.

(a) Transfer 50 ml aliquot to 500 ml Erlenmeyer flask, add 10 ml of HCl, heat to  $90^\circ$ , and titrate with the standard bromate soln as directed under 3(c), beginning with "titrate with the bromate soln." Or,

(b) Proceed as directed under (a) but make titration without heating soln.

From number of ml of bromate soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

29

## WATER-SOLUBLE ARSENIOS OXIDE—OFFICIAL

To 1 g of sample in liter Florence flask add 1 liter of recently boiled  $H_2O$  that has been cooled to  $32^\circ$ . Stopper flask and place in water bath kept at  $32^\circ$  by means of thermostat. Digest 24 hours, shaking hourly for 8 hours during this period. Filter thru dry filter and transfer 250 ml of filtrate to Erlenmeyer flask; add 4–5 g of  $NaHCO_3$  and titrate with the I soln, 3(b), to permanent blue color, using starch indicator, 3(e). Correct for quantity of I soln necessary to produce same color, using same reagents and volume. Calculate quantity of  $As_2O_3$  present and express results as percentage of water-soluble  $As_2O_3$ .

## TOTAL COPPER OXIDE

30

*Electrolytic Method—Official*

Treat 2 g of sample in beaker with 100 ml of  $H_2O$  and ca 2 g of  $NaOH$  and boil thoroly until all Cu is precipitated as  $Cu_2O$ . Filter, wash well with hot  $H_2O$ , dissolve precipitate in hot  $HNO_3$  (1+4), cool, transfer to 250 ml volumetric flask, and dilute to mark. Electrolyze aliquot of 50 or 100 ml, as directed under 14. Calculate to percentage of  $CuO$ .

31

*Volumetric Thiosulfate Method<sup>12</sup>—Official*

Determine Cu in aliquot of the  $HNO_3$  soln of  $Cu_2O$ , 30, by titrating with standard thiosulfate soln as directed under 15, and calculate to percentage of  $CuO$ .

## LEAD ARSENATE

32

## MOISTURE—OFFICIAL

(a) *Powder*.—Dry 2 g to constant weight at  $105$ – $110^\circ$  and report loss in weight as moisture.

(b) *Paste*.—Proceed as directed under (a), using 50 g. Grind dry sample to fine powder, mix well, transfer small portion to sample bottle, and again dry for 1–2 hours at  $105$ – $110^\circ$ . Use this anhydrous material for determination of total  $PbO$  and total As.

## TOTAL ARSENIC

33

*Method I—Official.—See 5 or 8.*

34

*Method II<sup>13</sup>—Official*

(Not applicable in presence of antimony.)

Dissolve 1 g of powdered sample with  $HNO_3$  (1+4) in porcelain casserole or evaporating dish, add 5 ml of  $H_2SO_4$ , and heat on hot plate to copious evolution of white fumes. Cool, add a little  $H_2O$ , and again evaporate until appearance of white fumes to assure removal of last trace of  $HNO_3$ . Wash into 200 ml volumetric flask with  $H_2O$ , cool, dilute to volume, and filter thru a dry filter. Transfer 100 ml of filtrate to Erlenmeyer flask and proceed as directed under 12, beginning with "add 1 g of KI." From number of ml of standard I soln used calculate percentage of total As in terms of  $As_2O_5$ .

35

TOTAL ARSENIOS OXIDE<sup>14</sup>—OFFICIAL

Weigh 2 g of powdered sample and transfer to 200 ml volumetric flask, add 100 ml of  $H_2SO_4$  (1+6), and boil 30 min. Cool, dilute to volume, shake thoroly, and filter thru dry filter. Nearly neutralize 100 ml of filtrate with  $NaOH$  soln, 3(d),



using a few drops of phenolphthalein indicator, II, 10(d). If neutral point is passed, make acid again with the dilute  $\text{H}_2\text{SO}_4$ . Continue as directed under 3(b), beginning "neutralize with  $\text{NaHCO}_3$ ." From number of ml of standard I soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

TOTAL ARSENIC OXIDE<sup>15</sup>—TENTATIVE

36

## REAGENTS

(a) *Potassium iodide soln.*—Dissolve 20 g of KI in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(b) *Standard thiosulfate soln.*—Prepare ca 0.05 *N* soln as follows: Dissolve 13 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in recently boiled and cooled  $\text{H}_2\text{O}$ , filter, and dilute to 1 liter with recently boiled and cooled  $\text{H}_2\text{O}$ . Standardize as follows:

Dissolve ca 0.7 g of  $\text{PbHAsO}_4$  in 50 ml of HCl in Erlenmeyer flask. If necessary to effect soln, heat on steam bath, keeping flask covered with watch-glass to prevent evaporation of acid. Cool to 20–25°, add 10 ml of the KI soln, (a), and 50 ml (or more if necessary to produce clear soln) of  $\text{NH}_4\text{Cl}$  soln, 26, and immediately titrate liberated I with the standard thiosulfate. When color becomes a faint yellow, dilute with ca 150 ml of  $\text{H}_2\text{O}$  and continue titration carefully, dropwise, until colorless, using starch indicator, 3(e), near end point. From weight of  $\text{PbHAsO}_4$  and number of ml of  $\text{Na}_2\text{S}_2\text{O}_3$  soln used calculate value of latter in terms of  $\text{As}_2\text{O}_5$  ( $\text{As}_2\text{O}_5$  in  $\text{PbHAsO}_4 = 33.11\%$ ).

Prepare pure  $\text{PbHAsO}_4$  by pouring a soln of  $\text{Pb}(\text{NO}_3)_2$  into a soln of  $\text{KH}_2\text{AsO}_4$ , which should be in excess. Collect precipitate by filtration, dissolve it in smallest possible quantity of boiling  $\text{HNO}_3$  (1+4), and pour soln into large quantity of  $\text{H}_2\text{O}$  (50–100 ml of the  $\text{HNO}_3$  soln in 2–3 liters of  $\text{H}_2\text{O}$ ). Collect precipitate by filtration and dry at 110°.

37

## DETERMINATION

Weigh 0.5 g of powdered sample and transfer to Erlenmeyer flask. Add 25–30 ml of HCl and evaporate to dryness on steam bath. Add 50 ml of HCl and proceed as directed under 36(b), beginning with "If necessary to effect soln, heat on steam bath." From number of ml of standard thiosulfate soln used calculate percentage of  $\text{As}_2\text{O}_5$ .

38

## WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12, and calculate results as  $\text{As}_2\text{O}_5$ .

## TOTAL LEAD OXIDE

39

Method I<sup>16</sup>—Official

Heat in 600 ml beaker on hot plate, 0.5 g of powdered sample and ca 25 ml of  $\text{HNO}_3$  (1+4). Remove any insoluble residue by filtration. Dilute to at least 400 ml, heat nearly to boiling, and add  $\text{NH}_4\text{OH}$  to slight precipitation, then  $\text{HNO}_3$  (1+9) to redissolve precipitate, adding 1–2 ml in excess. Pipet into this soln, kept almost boiling, 50 ml of hot 10%  $\text{K}_2\text{CrO}_4$  soln, stirring constantly. Decant while hot thru weighed Gooch crucible, previously heated to 140–150°, and wash several times by decantation and then on filter with boiling  $\text{H}_2\text{O}$  until washings are colorless. Dry the  $\text{PbCrO}_4$  at 140–150° to constant weight. From weight of  $\text{PbCrO}_4$  calculate percentage of  $\text{PbO}$ , using factor 0.6906. (The  $\text{PbCrO}_4$  precipitate may contain a small quantity of  $\text{PbHAsO}_4$ , which will cause slightly high results, but this error rarely amounts to more than 0.1–0.2%.)

40

*Method II<sup>17</sup>—Official*

(Not applicable in presence of calcium.)

Heat in porcelain evaporating dish or casserole on hot plate, 0.5 g of powdered sample and ca 25 ml of  $\text{HNO}_3$  (1+4). Remove any insoluble residue by filtration. Add 3 ml of  $\text{H}_2\text{SO}_4$  and evaporate on hot plate until appearance of white fumes. Cool, add few ml of  $\text{H}_2\text{O}$  (to decompose any nitro-sulfuric acid formed), and again heat to fuming. Proceed as directed under 13, beginning with "Cool, add 50 ml of  $\text{H}_2\text{O}$  and 100 ml of alcohol."

## CALCIUM ARSENATE

41

MOISTURE—OFFICIAL.—*See* 2.

42

TOTAL ARSENIC—OFFICIAL.—*See* 5 or 8.

43

TOTAL ARSENIOS OXIDE<sup>18</sup>—OFFICIAL

(a) *Not applicable in presence of nitrates.*—Weigh 1 g of sample, transfer to 500 ml Erlenmeyer flask, and dissolve in 100 ml of  $\text{HCl}$  (1+3). Heat to  $90^\circ$  and titrate with the standard bromate soln, 3(c), using 10 drops of the methyl orange indicator, 3(f). From number of ml of standard bromate soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

(b) *Applicable in presence of small quantities of nitrates.*—Proceed as directed under (a) except to make titration at room temp.

44

WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12, and calculate results as  $\text{As}_2\text{O}_5$ .

## TOTAL CALCIUM OXIDE

*Method I<sup>18</sup>—Official*

45

## REAGENTS

(a) *Ammonium oxalate soln.*—Dissolve 40 g of  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  in 1 liter of  $\text{H}_2\text{O}$ .

(b) *Standard potassium permanganate soln.*—Dissolve 3.161 g of  $\text{KMnO}_4$  in freshly distilled  $\text{H}_2\text{O}$  and dilute to 1 liter. Filter thru asbestos in Gooch crucible and allow to stand several days in dark place. To standardize, dissolve 0.25 g of pure  $\text{Na}_2\text{C}_2\text{O}_4$  in  $\text{H}_2\text{O}$ , add 25 ml of  $\text{H}_2\text{SO}_4$  (1+4), dilute to 200 ml, heat to ca  $70^\circ$ , and titrate with the  $\text{KMnO}_4$  soln until soln assumes faint pink color. From this titration calculate conc. of the  $\text{KMnO}_4$  soln, which should be ca 0.1 N.

46

## DETERMINATION

Dissolve 2 g of sample in 80 ml of acetic acid (1+3), transfer to 200 ml volumetric flask, dilute to volume, and filter thru dry filter. Transfer 50 ml aliquot to beaker, dilute to ca 200 ml, heat to boiling, and precipitate the Ca with the  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln. Allow beaker to stand 3 hours on steam bath, filter soln, and wash precipitate with hot  $\text{H}_2\text{O}$ . Dissolve precipitate in 200 ml of  $\text{H}_2\text{O}$  containing 25 ml of  $\text{H}_2\text{SO}_4$  (1+4), heat to ca  $70^\circ$ , and titrate with the  $\text{KMnO}_4$  soln. From number of ml of  $\text{KMnO}_4$  soln used calculate percentage of  $\text{CaO}$ .

47

*Method II<sup>18</sup>—Official*

(Not applicable in presence of lead.)

Weigh 2 g of sample; transfer to beaker, add 5 ml of  $\text{HBr}$  (ca 1.38 sp. gr.) and

15 ml of HCl, and evaporate to dryness under hood to remove As. Repeat treatment, add 20 ml of HCl, and again evaporate to dryness. Take up with H<sub>2</sub>O and a little HCl, filter into 200 ml volumetric flask, wash, and dilute to volume. Transfer 50 ml aliquot to beaker, add 10 ml of HCl and few drops of HNO<sub>3</sub>, boil, and make slightly alkaline with NH<sub>4</sub>OH. Let stand few minutes and filter. Dissolve precipitate in HCl (1+4), reprecipitate, filter thru same paper, and wash with hot H<sub>2</sub>O. To combined filtrates and washings add 20 ml of acetic acid (1+3) and adjust volume to ca 200 ml. Heat to boiling, precipitate with the (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub> soln, 45(a), and allow to stand 3 hours on steam bath. Filter, and wash with hot H<sub>2</sub>O. Ignite, and weigh as CaO; or dissolve precipitate in 200 ml of H<sub>2</sub>O containing 25 ml of H<sub>2</sub>SO<sub>4</sub> (1+4), heat to ca 70°, and titrate with the KMnO<sub>4</sub> soln, 45(b). From weight of CaO or number of ml of KMnO<sub>4</sub> soln used calculate percentage of CaO.

#### MAGNESIUM ARSENATE

48

MOISTURE—OFFICIAL.—*See* 2.

49

TOTAL ARSENIC—OFFICIAL.—*See* 5 or 8.

50

TOTAL ARSENIUS OXIDE—OFFICIAL.—*See* 35.

51

WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12, and calculate as As<sub>2</sub>O<sub>3</sub>.

#### ZINC ARSENITE

52

MOISTURE—OFFICIAL.—*See* 2.

53

TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, and calculate as As<sub>2</sub>O<sub>3</sub>.

#### TOTAL ARSENIUS OXIDE

54

*Method I*<sup>18</sup>—Official

(a) Weigh 2 g of sample and transfer to beaker. Dissolve in 80 ml of HCl (1+4), wash into 200 ml volumetric flask, and dilute to volume. Thoroly mix soln and filter thru dry filter. Transfer 25 ml aliquot to 500 ml Erlenmeyer flask, add 20 ml of HCl, and dilute to 100 ml. Heat to 90° and titrate with the standard bromate soln, 3(c). Or,

(b) Proceed as directed under (a) without heating soln.

55

*Method II*—Official

Proceed as directed under 27, using appropriate Zn salt for blank determination.

56

WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12, and calculate results as As<sub>2</sub>O<sub>3</sub>.

57

TOTAL ZINC OXIDE<sup>18</sup>—OFFICIAL

Transfer 25 ml aliquot of soln prepared for determination of total As<sub>2</sub>O<sub>3</sub>, 54, to beaker and add 5 ml of HCl. If there is much Fe present, reduce it by adding a little NaHSO<sub>3</sub> and heating on steam bath until odor of SO<sub>2</sub> has practically disappeared. Cool, dilute to ca 100 ml, and proceed as directed under 17, beginning with "add 35–40 ml of the Hg-thiocyanate reagent with vigorous stirring."

## COPPER CARBONATE

## COPPER OXIDE

58

*Electrolytic Method—Official*

Weigh 0.5 g of sample, transfer to 150 ml Pt dish or 150 ml beaker, and dissolve in 25 ml of  $\text{HNO}_3$  (1+4). Dilute to ca 100 ml and determine Cu by electrolysis, as directed under 14, beginning "electrolyze, using rotating anode and current of ca 3 amperes."

59

*Thiosulfate Volumetric Method—Official*

Dissolve 0.25–0.5 g of sample in 25 ml of  $\text{HNO}_3$  (1+4), dilute to ca 50 ml, and proceed as directed under 15, beginning "add  $\text{NH}_4\text{OH}$  in excess."

## BORDEAUX MIXTURE

60

## MOISTURE—OFFICIAL

(a) *Powder*.—Dry 2 g to constant weight at 105–110°. Report loss as moisture.

(b) *Paste*.—Heat ca 100 g in oven at 90–100° until dry enough to powder readily and note loss in weight. Powder this partially dried sample and determine remaining moisture in 2 g as directed under (a). Determine  $\text{CO}_2$  as directed under 62, both in original paste and in this partially dried sample. Calculate total moisture by following formula:

$$M = a + \frac{(100 - a)(b + c)}{100} - d, \text{ in which}$$

$M$  = % of total moisture in original paste;

$a$  = % of loss in weight of original paste during first drying;

$b$  = % of loss in weight of partially dried paste during second drying;

$c$  = % of  $\text{CO}_2$  remaining in partially dried paste after first drying; and

$d$  = % of total  $\text{CO}_2$  in original paste.

CARBON DIOXIDE<sup>18</sup>—OFFICIAL

61

## APPARATUS

Use a 200 ml Erlenmeyer flask closed with 2-holed stopper; in one hole fit a dropping funnel, allowing stem to extend almost to bottom of flask, and thru other hole pass outlet of a condenser that is inclined upward at angle of 30° from horizontal. Connect upper end of condenser with a  $\text{CaCl}_2$  tube, which in turn is connected with a double U-tube filled in middle with pumice fragments, previously saturated with  $\text{CuSO}_4$  soln (20%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and subsequently dehydrated, and with  $\text{CaCl}_2$  at either end. Connect two weighed U-tubes for absorbing the  $\text{CO}_2$ , the first filled with porous soda-lime, and the second,  $\frac{1}{3}$  with soda-lime and  $\frac{2}{3}$  with  $\text{CaCl}_2$ , placing the  $\text{CaCl}_2$  at exit end of train. Attach a Geissler bulb, partly filled with  $\text{H}_2\text{SO}_4$ , to last U-tube to show rate of gas flow, and connect an aspirator with Geissler bulb to draw air thru apparatus. Connect an absorption tower filled with soda-lime to mouth of dropping funnel to remove  $\text{CO}_2$  from the air entering apparatus.

62

## DETERMINATION

Weigh 2 g of powder or 10 g of paste into the Erlenmeyer flask and add ca 20 ml of  $\text{H}_2\text{O}$ . Attach flask to apparatus, omitting the 2 weighed U-tubes, and draw  $\text{CO}_2$ -

free air thru apparatus until it displaces original air. Attach weighed U-tubes as directed under 61, close stopcock of dropping funnel, pour into it 50 ml of HCl (1+4), reconnect with soda-lime tower, and allow the acid to flow into Erlenmeyer flask, slowly if there is much CO<sub>2</sub>, rapidly if there is little. When effervescence diminishes, place low Bunsen flame under flask and start flow of H<sub>2</sub>O thru condenser, allowing slow current of air to flow thru apparatus at same time. Maintain a steady but quiet ebullition and a slow air current thru apparatus. Boil a few minutes after the H<sub>2</sub>O has begun to condense in condenser, remove flame, and continue aspiration of air at rate of about 2 bubbles per second until apparatus is cool. Disconnect weighed absorption tubes, cool in balance case, and weigh. The increase in weight is CO<sub>2</sub>.

#### COPPER

63

#### *Electrolytic Method—Official*

Dissolve 2 g of powdered sample in 25 ml of HNO<sub>3</sub> (1+4), dilute to 100 ml, and electrolyze, using rotating spiral anode and current of about 3 amperes, as directed under 14, beginning "Wash into weighed 150 ml Pt dish."

64

#### *Thiosulfate Volumetric Method—Official*

Dissolve 2 g of powdered sample in ca 25 ml of HNO<sub>3</sub> (1+4), dilute to 50 ml, add NH<sub>4</sub>OH in excess, and heat. Without removing precipitate that has formed, boil off excess of NH<sub>3</sub>, add 3–4 ml of acetic acid, cool, add 10 ml of 30% KI soln., and titrate as directed under 15, beginning "titrate with standard thiosulfate soln."

### BORDEAUX MIXTURE WITH PARIS GREEN

65

#### MOISTURE—OFFICIAL.—See 60.

66

#### CARBON DIOXIDE—OFFICIAL.—See 61.

67

#### TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of sample and calculating results as As<sub>2</sub>O<sub>3</sub>.

68

#### TOTAL ARSENIOS OXIDE—OFFICIAL

Proceed as directed under 27, using 0.5–1.0 g of sample.

69

#### WATER-SOLUBLE ARSENIOS OXIDE—OFFICIAL

Proceed as directed under 29, using 2 g of sample and slightly acidifying aliquot used with HCl (1+4) before adding the excess of NaHCO<sub>3</sub>.

#### COPPER

70

#### *Electrolytic Method I—Official.—See 14.*

71

#### *Electrolytic Method II—Official*

(Short Method.)

Dissolve 2 g of powdered sample in 150 ml beaker with 5 ml of HNO<sub>3</sub>, add 25 ml of 3% soln of H<sub>2</sub>O<sub>2</sub>, and warm on steam bath 5–10 min. Add 25 ml more of H<sub>2</sub>O<sub>2</sub> soln, dilute to 100 ml, and electrolyze, using weighed gauze cathode, a rotating paddle anode, and current of 2–3 amperes. At end of ca 20 min., add 15–20 ml more of the H<sub>2</sub>O<sub>2</sub> soln. After all Cu is deposited (which should not require more than 45 min.) and while current is still flowing, wash deposit with H<sub>2</sub>O by siphoning.

Interrupt current, rinse with alcohol, dry few minutes in oven, weigh, and calculate percentage of Cu. (Do not pass the current for more than 5–10 min. after all Cu is deposited without adding more of the  $\text{H}_2\text{O}_2$  soln.)

72 *Thiosulfate Volumetric Method—Official.—See 15.*

#### BORDEAUX MIXTURE WITH LEAD ARSENATE

73 MOISTURE—OFFICIAL.—*See 60.*

74 CARBON DIOXIDE—OFFICIAL.—*See 61.*

75 TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of sample and calculating results as  $\text{As}_2\text{O}_5$ .

76 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12 and calculate results as  $\text{As}_2\text{O}_5$ .

#### COPPER

77 *Electrolytic Method—Official.—See 14.*

78 *Thiosulfate Volumetric Method—Official.—See 15.*

79 LEAD OXIDE—OFFICIAL.—*See 13.*

#### LEAD OXIDE AND COPPER

*Electrolytic Method<sup>20</sup>—Official*

80 APPARATUS

*Electrodes.*—Cathode, a cylindrical Pt electrode, either gauze or plate, ca 50 mm high and 25 mm in diameter. Anode, gauze or plate, ca 50 mm high and 50 mm in diameter. This electrode should be sandblasted.

81 DETERMINATION

Weigh 1 g of powdered sample and transfer to 250 ml beaker. Add 15 ml of HCl and 5 ml of HBr, and evaporate to dryness on steam bath. Repeat treatment, and finally, to remove last traces of As, add 20 ml of the HCl and again evaporate to dryness.

To residue add 25 ml of  $\text{H}_2\text{O}$  and 15 ml of  $\text{HNO}_3$  and heat to boiling. Cautiously boil until most of bromides and some of chlorides are expelled (characterized by changes in color, first from brown to green, and then to blue). Evaporate to dryness on steam bath. Add 10 ml of  $\text{H}_2\text{O}$  and 15 ml of  $\text{HNO}_3$ , and again evaporate to dryness. Take up in 50 ml of  $\text{H}_2\text{O}$  and 12 ml of  $\text{HNO}_3$  and heat until all salts are in solution. (It is not necessary to filter off any siliceous material that may be present.) Dilute to 200 ml and electrolyze overnight, using current of 0.15 ampere and potential of 1.5–2 volts.

Add 15–20 ml of  $\text{H}_2\text{O}$  to electrolyte and continue to use current a few minutes. If there is no further deposition on newly exposed surfaces of electrodes, wash them several times with  $\text{H}_2\text{O}$  without breaking current. Finally break current and wash once with methyl or ethyl alcohol. Dry electrodes in oven at 105–110° 1 hour. Increase in weight of cathode represents the Cu present in sample, and increase in weight of anode represents the lead as  $\text{PbO}_2$ . From increased weight of cathode,

calculate percentage of Cu. As the  $\text{PbO}_2$  is not completely anhydrous, multiply weight found by factor 0.9267, and calculate percentage of  $\text{PbO}$ .

### BORDEAUX MIXTURE WITH CALCIUM ARSENATE

82 MOISTURE—OFFICIAL.—See 60.

83 CARBON DIOXIDE—OFFICIAL.—See 62.

84 TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of sample, and calculating results as  $\text{As}_2\text{O}_5$ .

85 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 12 and calculate results as  $\text{As}_2\text{O}_5$ .

### COPPER

86 *Electrolytic Method I—Official.—See 14.*

87 *Electrolytic Method II—Official.—See 71.*

88 *Thiosulfate Volumetric Method—Official.—See 15.*

### SODIUM AND POTASSIUM CYANIDES

#### CYANOGEN<sup>21</sup>—OFFICIAL

89 REAGENT

*Silver nitrate soln.*—0.1 N. Standardize against pure NaCl by titration, using chromate indicator.

90 DETERMINATION

Break sample into small lumps in mortar (do not grind). Weigh quickly ca 5 g in weighing bottle and wash into 500 ml volumetric flask containing ca 200 ml of  $\text{H}_2\text{O}$ . Add a little  $\text{PbCO}_3$  to precipitate any sulfides that may be present, dilute to mark with  $\text{H}_2\text{O}$ , mix thoroly, and filter thru dry filter. Transfer 50 ml aliquot to 400 ml beaker; add 200 ml of  $\text{H}_2\text{O}$ , 5 ml of NaOH soln (100 g to 1 liter of  $\text{H}_2\text{O}$ ), and 10 drops of saturated KI soln (or a few crystals); and titrate to faint opalescence with the  $\text{AgNO}_3$  soln. (In making this titration, it is advantageous to have the beaker over a black surface.) From number of ml of 0.1 N  $\text{AgNO}_3$  soln used calculate percentage of CN. The reaction is represented by the equation:  $2\text{NaCN} + \text{AgNO}_3 = \text{NaCN} \cdot \text{AgCN} + \text{NaNO}_3$ ; hence 1 ml of 0.1 N  $\text{AgNO}_3$  soln = 0.005204 g of CN.

#### CHLORINE<sup>22</sup>

##### *Method I—Official*

91 REAGENTS

(a) *Ammonium or potassium thiocyanate soln.*—0.1 N. Adjust by titrating against the 0.1 N  $\text{AgNO}_3$  soln, 89.

(b) *Ferric indicator.*—A saturated soln of ferric ammonium alum from which brown color has been removed by addition of few drops of  $\text{HNO}_3$ .

92 DETERMINATION

Transfer a 50 ml aliquot of prepared soln, 90, to beaker, dilute with equal volume

of  $\text{H}_2\text{O}$ , add 1–2 ml of 40% chloride-free  $\text{HCHO}$  soln, stir well, and let stand 15 min. Acidify with 5 ml  $\text{HNO}_3$  (1+1), add measured volume of 0.1  $N$   $\text{AgNO}_3$  soln, 89, sufficient to give an excess, stir well, filter, wash, and titrate excess of Ag in combined filtrate and washings with the 0.1  $N$  thiocyanate soln, using the ferric indicator. From number of ml of 0.1  $N$   $\text{AgNO}_3$  soln, less number of ml of thiocyanate soln used, calculate percentage of Cl.

93

*Method II<sup>22</sup>—Official*

Transfer 50 ml aliquot of prepared soln, 90, to distilling flask, dilute to 100–150 ml, acidify with slight excess of acetic acid, and distil, passing vapors thru a condenser, the delivery end of which dips into a soln of  $\text{NaOH}$ , to absorb the  $\text{HCN}$ . After all the  $\text{HCN}$  has been driven off (50 ml of distillate), wash liquid remaining in distilling flask into beaker, add 5 ml of  $\text{HNO}_3$  (1+1) and then a measured volume of 0.1  $N$   $\text{AgNO}_3$  soln, 89, sufficient to give an excess. Stir well, filter, wash, and titrate excess of Ag in combined filtrate and washings with thiocyanate soln, 91(a), using ferric indicator, 91(b). From number of ml of 0.1  $N$   $\text{AgNO}_3$  soln, less number of ml of 0.1  $N$  thiocyanate soln used, calculate percentage of Cl.

## CALCIUM CYANIDE

CYANOGEN<sup>23</sup>—OFFICIAL

94

## REAGENT

*Soda-lead.*—Dissolve 20 g of Pb acetate in  $\text{H}_2\text{O}$ , dilute to 1 liter, and add 200 g of chloride-free  $\text{Na}_2\text{CO}_3$ .

95

## DETERMINATION

Place ca 200 ml of  $\text{H}_2\text{O}$  in 500 ml volumetric flask and carefully dry neck of flask. Weigh ca 5 g of sample in weighing bottle and transfer to flask with least possible exposure to air. Wash mixture down into flask and mix by whirling until soln is complete and the small quantity of  $\text{CaC}_2$  has been decomposed. Add 25 ml of the soda-lead, or a quantity sufficient to remove sulfides; close flask with rubber stopper; and shake thoroly, preferably 30 min. Dilute to mark, mix, and filter thru dry filter. Transfer 50 ml aliquot to 400 ml beaker and proceed as directed under 90, beginning "Add 200 ml of  $\text{H}_2\text{O}$ ." 1 ml of 0.1  $N$   $\text{AgNO}_3$  soln = 0.005204 g of CN. To obtain percentage of  $\text{Ca}(\text{CN})_2$ , multiply percentage of CN by factor 1.7702.

CHLORINE<sup>24</sup>

96

*Method I—Official*

Transfer 50 ml aliquot of prepared soln, 95, to beaker, and proceed as directed under 92.

97

*Method II<sup>23</sup>—Official*

Transfer 50 ml aliquot of prepared soln, 95, to distilling flask, and proceed as directed under 93.

## SOAP

MOISTURE<sup>24</sup>

98

*Xylene Distillation Method—Official*

Weigh ca 20 g of sample into 300–500 ml flask; add 50 ml of xylene (technical grade is satisfactory); and, to prevent foaming, add ca 10 g of lump rosin (do not



use powdered). Distil into Dean and Stark type distilling tube receiver<sup>25</sup> and continue distillation until no more  $H_2O$  collects in receiver. Allow contents of tube to cool to room temp., read volume of  $H_2O$  under the xylene in the tube, and from this volume calculate percentage of  $H_2O$ .

99

POTASSIUM AND SODIUM<sup>26</sup>—OFFICIAL

Dissolve ca 5 g of the soap in  $H_2O$ , decompose with  $HCl$  (1+4), filter off the  $H_2O$ , and wash the fat with cold  $H_2O$ . Determine both K and Na in filtrate as directed under XII, 14 and 15.

## MINERAL OILS

100 UNSULFONATABLE RESIDUE<sup>27</sup>—OFFICIAL

Pipet 5 ml of the oil into Babcock cream bottle ca 15 cm (6") long (either the 9 g 50% or the 18 g 30% type). To reduce viscosity of heavy oils, warm pipet after preliminary draining by drawing it several times thru flame of Bunsen burner and drain thoroly. If greater accuracy is desired, weigh measured charge and calculate its exact volume from weight and sp. gr. of the oil. Add slowly 20 ml of 38 N  $H_2SO_4$ , VIII, 18, gently shaking or rotating bottle and taking care that temp. does not rise above 60°. Cool in ice  $H_2O$  if necessary. When mixture no longer develops heat on shaking, agitate thoroly, place bottle in water bath, and heat at 60–65° for 10 min., keeping contents of bottle thoroly mixed by shaking vigorously 20 seconds at 2 min. intervals. Remove bottle from bath and fill with  $H_2SO_4$  until oil rises into graduated neck. Centrifuge 5 min. (or longer if necessary to obtain a constant volume of oil) at 1200–1500 r.p.m. Read volume of unsulfonatable residue from graduations on neck of bottle and, to convert to ml, multiply reading from the 9 g 50% bottle by 0.1 and that from the 18 g 30% bottle by 0.2. From result obtained calculate percentage by volume of unsulfonatable oil.

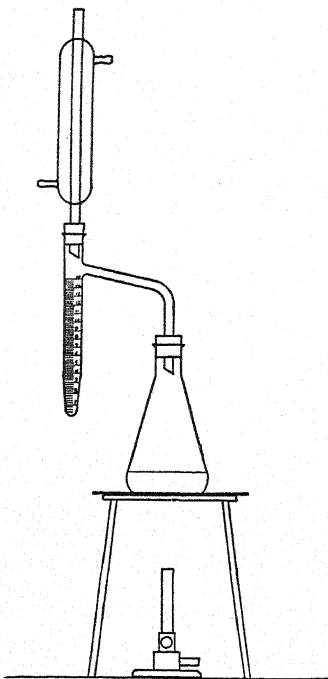


FIG. 9.—DEAN AND STARK DISTILLING TUBE RECEIVER

## MINERAL OIL—SOAP EMULSIONS

WATER<sup>28</sup>

101

*Xylene Distillation Method—Official*

Weigh ca 25 g of sample and proceed as directed in 98, except to use a smaller quantity of rosin.

102

TOTAL OIL<sup>29</sup>—OFFICIAL

Weigh ca 10 g of sample into Babcock cream bottle, 100. Dilute with ca 10 ml of hot  $H_2O$  and add 5–10 ml of  $H_2SO_4$  (1+1). Set bottle in hot water bath ca 5 min. to hasten separation of oil, add sufficient saturated  $NaCl$  soln to bring oil layer within graduations on neck of bottle, whirl at rate of 1200 r.p.m. for 5 min., and

allow to cool. Read volume of oil layer, determine its density, and from these values calculate its weight and percentage. From this percentage value, deduct percentage of fatty acids (and phenols if present) determined separately, to obtain percentage of oil.

103

SOAP<sup>28</sup>—OFFICIAL

(Error will result if apparent molecular weight of fatty acids varies appreciably from that of oleic acid.)

Weigh 20 g of sample into a separatory funnel, add 60 ml of petroleum benzin, and extract mixture once with 20 ml and four times with 10 ml of 50% alcohol. Break emulsion if necessary with 1 or 2 ml of a 20% soln of NaOH, allowing soln to run down side of separatory funnel, which is then gently twirled and allowed to stand a few minutes. Draw off alcoholic layers and wash successively thru petroleum benzin contained in 2 other separatory funnels. Combine alcoholic extracts in beaker and evaporate on steam bath to remove alcohol. Dissolve residue in ca 100 ml of H<sub>2</sub>O made alkaline with NaOH. Transfer to a separatory funnel, acidify with HCl or H<sub>2</sub>SO<sub>4</sub>, extract 3 times with ether, and wash ether extracts twice with H<sub>2</sub>O. Combine ether extracts, evaporate in weighed beaker on steam bath, and weigh as fatty acids. From weight of fatty acids calculate percentage of soap in sample as Na- or K-oleate.

104

## UNSULFONATABLE RESIDUE—OFFICIAL

Using 5 ml of recovered oil, proceed as directed under 100.

105

ASH<sup>29</sup>—OFFICIAL

Evaporate 10 g of sample, or more if necessary, in Pt dish; ignite, and leach charred mass with H<sub>2</sub>O. Ignite residue, add leachings, evaporate to dryness, ignite, and weigh. From this weight calculate percentage of ash. Test ash for Cu, Ca, CaF<sub>2</sub>, etc.

## SODA LYE

CARBONATE AND HYDROXIDE<sup>31</sup>—OFFICIAL

106

## REAGENTS

(a) *Phenolphthalein indicator*.—Dissolve 1 g of phenolphthalein in 100 ml of neutralized alcohol.

(b) *Barium chloride soln*.—Dissolve 100 g of BaCl<sub>2</sub>·2H<sub>2</sub>O and dilute to 1 liter.

107

## DETERMINATION

Weigh ca 10 g of sample from weighing bottle, dissolve in CO<sub>2</sub>-free H<sub>2</sub>O, and dilute to definite volume. Titrate aliquot of this soln with the 0.5 N HCl, II, 19(a), using the methyl orange indicator, 3(f), and note the total alkalinity thus found. Transfer an equal aliquot to volumetric flask and add enough of the BaCl<sub>2</sub> soln to precipitate all the carbonate, avoiding any unnecessary excess. Dilute to mark with CO<sub>2</sub>-free H<sub>2</sub>O, stopper, shake, and set aside. When liquid becomes clear, pipet off one-half and titrate with the 0.5 N HCl, using the phenolphthalein indicator; ml of 0.5 N acid required for this titration × 2 = ml of 0.5 N acid equivalent to NaOH present in original aliquot. The difference between this figure and the ml of 0.5 N HCl required for total alkalinity represents the ml of 0.5 N acid equivalent to the Na<sub>2</sub>CO<sub>3</sub> present in aliquot. Calculate percentages of Na<sub>2</sub>CO<sub>3</sub> and NaOH.

## TOBACCO AND TOBACCO EXTRACT

## NICOTINE

*Silicotungstic Acid Method*<sup>32</sup>—Official

108

## REAGENT

*Silicotungstic acid soln.*—Dissolve 120 g of silicotungstic acid ( $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 \cdot 22\text{H}_2\text{O}$ ) in  $\text{H}_2\text{O}$  and dilute to 1 liter. (This acid should be white or pale yellow crystals, free from green color. The solution should be free from cloudiness and green color. Of the several silicotungstic acids,  $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 10\text{WO}_3 \cdot 3\text{H}_2\text{O}$  and  $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 \cdot 20\text{H}_2\text{O}$  do not give crystalline precipitates with nicotine and should not be used.)

109

## DETERMINATION

Weigh a quantity of the preparations that will contain preferably 0.1–1.0 g of nicotine. If sample contains very little nicotine (ca 0.1%) do not increase quantity to point where it interferes with distillation. Wash with  $\text{H}_2\text{O}$  into 500 ml Kjeldahl flask; and, if necessary, add a little paraffin to prevent frothing and a few small pieces of pumice to prevent bumping. Add a slight excess of  $\text{NaOH}$  soln, using phenolphthalein indicator, and close flask with rubber stopper thru which passes stem of trap bulb and inlet tube for steam. Connect by means of trap bulb to well-cooled condenser, the lower end of which dips below surface of 10 ml of  $\text{HCl}$  (1+4) in suitable receiving flask. Distil rapidly with current of steam. When distillation is well under way heat distillation flask to reduce volume of liquid as far as practicable without bumping or undue separation of insoluble matter. Distil until a few ml of distillate shows no cloud or opalescence when treated with drop of the silicotungstic acid and drop of  $\text{HCl}$  (1+4). Confirm alkalinity of residue in distillation flask with phenolphthalein indicator. Test filtrate with methyl orange to confirm its acidity. Make distillate, which may amount to 1000–1500 ml, to convenient volume (soln may be concentrated on steam bath without loss of nicotine); mix well; and pass through dry filter if not clear. Pipet aliquot containing ca 0.1 g of nicotine into beaker (if samples contain very small quantities of nicotine, an aliquot containing as little as 0.01 g of nicotine may be used); add to each 100 ml of liquid 3 ml of  $\text{HCl}$  (1+4), and 1 ml of silicotungstic acid for each 0.01 g of nicotine supposed to be present. Stir thoroly and let stand overnight at room temp. Before filtering, stir precipitate to see that it settles quickly and is in crystalline form, filter on an ashless filter, and wash with  $\text{HCl}$  (1+1000) at room temp. Continue washing for 2 or 3 fillings of filter after no more opalescence appears when a few ml of fresh filtrate is tested with a few drops of nicotine distillate. Transfer paper and precipitate to weighed Pt crucible, dry carefully, and ignite until all C is destroyed. Finally heat over Meker burner for not more than 10 min. Weight of residue  $\times 0.1140$  = weight of nicotine present in aliquot.

## DERRIS AND CUBE POWDER

## ROTENONE

110

*Crystallization Method*<sup>33</sup>—Official, first action

Weigh 30 g (if sample contains more than 7% rotenone use a quantity that will give 1.0–1.5 g of rotenone in the 200 ml aliquot) of finely powdered root and 10 g of decolorizing carbon into 500 ml glass-stoppered Erlenmeyer flask. Add 300 ml of  $\text{CHCl}_3$  measured at definite room temp.; place flask on shaking machine and

fasten stopper securely. Agitate vigorously for not less than 4 hours, preferably interrupting shaking with overnight rest (or flask may be shaken continuously overnight). Remove flask from machine and allow to cool in refrigerator for at least an hour. Filter mixture rapidly into suitable flask, using fluted paper without suction and keeping funnel covered with watch-glass to avoid loss from evaporation. Stopper flask and adjust temp. of filtrate to that of original  $\text{CHCl}_3$ .

Transfer exactly 200 ml of this soln to 500 ml Pyrex Erlenmeyer flask and distil until only ca 25 ml remains in flask. Transfer extract to 125 ml Erlenmeyer flask, using  $\text{CCl}_4$  to rinse out the 500 ml flask. Evaporate almost to dryness on steam bath in current of air. Then remove remainder of solvent under reduced pressure, heating cautiously on steam bath when necessary to hasten evaporation (suction may be applied directly to flask). Dissolve extract in 15 ml of hot  $\text{CCl}_4$  and again, in similar manner, remove all solvent. Repeat with another 10–15 ml portion of hot  $\text{CCl}_4$ . (This treatment removes all  $\text{CHCl}_3$  from the resins. The  $\text{CHCl}_3$  extract is usually completely soluble in  $\text{CCl}_4$ . If small quantities of insoluble material are present, the purification procedure described later will eliminate them. However, if large quantity of insoluble residue should remain when extract is dissolved in first portion of  $\text{CCl}_4$ , it should be filtered off and thoroly washed with further portions of hot solvent, after which the filtered soln plus washings should be treated as directed above for removal of  $\text{CHCl}_3$ .)

Add exactly 25 ml of  $\text{CCl}_4$  and heat gently to completely dissolve extract. Cool flask in ice bath several minutes and seed with a few crystals of rotenone- $\text{CCl}_4$  solvate if necessary. Stopper flask and swirl until crystallization is apparent. If at this stage only a small quantity of crystalline material separates, add an accurately weighed quantity of pure rotenone estimated to be sufficient to assure that final result, expressed as pure rotenone, is at least 1 g. Then warm to effect complete soln, and again induce crystallization. At same time prepare saturated soln of rotenone in  $\text{CCl}_4$  for washing. Place flasks containing extract and washing soln in ice bath capable of maintaining temp. of  $0^\circ$  and allow to remain overnight.

After 17–18 hours in ice bath, rapidly filter extract thru weighed Gooch crucible fitted with disk of filter paper, removing flask from ice bath only long enough to pour each fraction of extract into crucible. Rinse residue of crystalline material from flask and wash under suction with sufficient of the ice-cold saturated soln (usually 10–12 ml) to remove excess mother liquor. Allow crucible to remain under suction ca 5 min. and then dry to constant weight at  $40^\circ$  (ca an hour). The weight obtained is "crude rotenone- $\text{CCl}_4$  solvate."

Break up contents of crucible with spatula, mix thoroly, and weigh 1 g into 50 ml Erlenmeyer flask. Add 10 ml of alcohol that has previously been saturated with rotenone at room temp., swirl flask a few minutes, stopper tightly, and set aside at least 4 hours, preferably overnight, at the same temp. Filter on weighed Gooch crucible fitted with disk of filter paper. Rinse crystals from flask and wash under suction with soln of ethyl alcohol saturated with rotenone at temp. of recrystallization (ca 10 ml will usually be required). Allow crucible to remain under suction 3–5 min. and then dry at  $105^\circ$  to constant weight, which should be effected in 1 hour.

Multiply weight, expressed in grams, by weight of crude rotenone- $\text{CCl}_4$  solvate, and to product add 0.07 g, which represents correction for rotenone held in soln in the 25 ml of  $\text{CCl}_4$  used in crystallization. If any pure rotenone has been added, subtract its weight from value obtained. This gives weight of pure rotenone contained in aliquot of extract, representing 20 g of sample.

*Alternative extraction procedure.*—If sample is one in which ratio of rotenone to total extract is greater than 40%, use quantity sufficient to contain 1.0–1.5 g of rotenone and successively extract four times with  $\text{CHCl}_3$ , using 200 ml each for the

second to fourth extractions. Filter after each extraction and return marc to flask for extraction with fresh solvent. Finally combine extracts, evaporate almost to dryness, and proceed as directed above, beginning at point where aliquot has been evaporated almost to dryness.

111

## TOTAL ETHER EXTRACT—OFFICIAL, FIRST ACTION

Extract 5 g of finely powdered root in a Soxhlet or other efficient extraction apparatus with ethyl ether for 48 hours. After extraction, concentrate extract and filter off any insoluble material that may be present. Receive filtrate in tared beaker. evaporate off ether on steam bath, and dry in oven at 105° to constant weight.

## PYRETHRUM POWDER

## PYRETHRIN I

*Mercury Reduction Method<sup>24</sup>—Official, first action*

112

## REAGENTS

(a) *Denigès' reagent*.—Mix 5 g of yellow HgO with 40 ml of H<sub>2</sub>O and, while stirring, slowly add 20 ml of H<sub>2</sub>SO<sub>4</sub>; then add another 40 ml portion of H<sub>2</sub>O and stir until completely dissolved. Test for absence of mercurous Hg by adding a few drops of (b) to 10 ml and titrating with (c) as directed under "Determination," beginning "Add 30 ml of HCl."

(b) *Iodine monochloride soln*.—Dissolve 10 g of KI and 6.44 g of KIO<sub>3</sub> in 75 ml of H<sub>2</sub>O; add 75 ml of HCl and 5 ml of CHCl<sub>3</sub> in glass-stoppered bottle and adjust to faint I color (in CHCl<sub>3</sub>) by adding dilute KI or KIO<sub>3</sub> soln. If there is much I set free, use a stronger soln of KIO<sub>3</sub> than 0.01 M at first, making final adjustment with 0.01 M soln. Keep in dark cupboard and readjust when necessary.

(c) *Standard potassium iodate soln*.—0.01 M. Dissolve 2.14 g of pure KIO<sub>3</sub>, previously dried at 105°, in H<sub>2</sub>O and dilute to 1 liter. 1 ml of this soln = 0.0044 g of Pyrethrin I, and needs no further standardization.

113

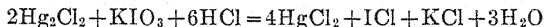
## DETERMINATION

Extract quantity of sample that will contain 20–75 mg of Pyrethrin I (12.5–20 g) in Soxhlet or other efficient extraction apparatus 7 hours with petroleum benzin, and evaporate petroleum benzin on water bath, heating no longer than necessary to remove solvent. Do not pass current of air thru flask during evaporation.

Add 15–20 ml of 0.5 N alcoholic NaOH soln to flask containing pyrethrum extract, connect to reflux condenser and boil gently 1–1.5 hours. Transfer to 600 ml beaker and add sufficient H<sub>2</sub>O to bring volume to 200 ml. Add a few glass beads or preferably use boiling tube, and boil down to 150 ml. Transfer to 250 ml volumetric flask, add 1 g of filter-cel and 10 ml of 10% BaCl<sub>2</sub> soln. Do not shake before making to volume. Make to volume, mix thoroly, filter off 200 ml, neutralize with H<sub>2</sub>SO<sub>4</sub> (1+4), and add 1 ml in excess, using 1 drop of phenolphthalein as indicator. (If necessary to have soln stand overnight at this point, it should be left in alkaline condition.) Filter thru 7 cm filter paper that has been coated lightly with suspension of filter-cel in H<sub>2</sub>O, on Büchner funnel and wash several times with H<sub>2</sub>O. Transfer into 500 ml separatory funnel and extract with two 50 ml portions of petroleum benzin. Wash extracts with 2 or 3 10 ml portions of H<sub>2</sub>O, and filter petroleum benzin extract thru plug of cotton into clean 250 ml separatory funnel. Wash cotton with 5 ml of petroleum benzin. Extract petroleum benzin with 5 ml of 0.1 N NaOH, shaking vigorously. Draw off aqueous layer into 100 ml beaker, wash petroleum

benzin with 5 ml of H<sub>2</sub>O or with additional 5 ml of 0.1 N NaOH, and add this to the beaker. Add 10 ml of Denigès' reagent to beaker and let stand 1 hour. Add 20 ml of alcohol to beaker and precipitate HgCl with 3 ml of saturated NaCl soln. Warm to ca 60°, and filter thru small filter paper, transferring all precipitate to filter paper, and wash with 10 ml or more of hot alcohol. Wash with two or more 10 ml portions of hot CHCl<sub>3</sub>, and place filter paper and contents in 250 ml glass-stoppered Erlenmeyer flask. Add 30 ml of HCl and 20 ml of H<sub>2</sub>O to flask and cool; add 6 ml of CHCl<sub>3</sub> or CCl<sub>4</sub> and 1 ml of ICl soln and titrate with the iodate soln, shaking vigorously after each addition, until there is no iodine color in CHCl<sub>3</sub> layer. From number of ml of the standard iodate soln used in titration calculate percentage of Pyrethrin I in sample.

KIO<sub>3</sub> reacts with mercurous Hg to form mercuric Hg and I. Further addition of iodate in presence of HCl oxidizes I to ICl.



Addition of ICl does not change volume relationship between mercurous Hg and iodate soln and aids in determining end point in titration of small quantities of Hg. The end point is taken when red color disappears from CHCl<sub>3</sub> layer. The end point is not permanent, therefore titration should be completed rapidly with vigorous shaking after each addition of iodate.

## 114

PYRETHRIN II<sup>35</sup>

Filter, if necessary, aqueous residue from petroleum benzin extraction in 113 thru Gooch crucible. Concentrate filtrate to ca 50 ml, transfer to separatory funnel, and neutralize with NaHCO<sub>3</sub>. Extract twice with CHCl<sub>3</sub> and wash CHCl<sub>3</sub> extract thru ca 15 ml of H<sub>2</sub>O in each of two separatory funnels. Combine aqueous soln and washings, acidify strongly with HCl (ca 8 ml), saturate with NaCl, adding cautiously at first to prevent excessive ebullition of CO<sub>2</sub>, and extract with 50 ml of ethyl ether. Draw off aqueous layer into a second separatory funnel and extract again with 50 ml of ether. Continue this extraction and drawing off of aqueous layer, using 35 ml for third and fourth extractions. Wash the four ether extracts successively with 10 ml of H<sub>2</sub>O, and repeat with second successive washing with another 10 ml of H<sub>2</sub>O. Combine ether solns, draw off any H<sub>2</sub>O that separates, and filter thru plug of cotton into 500 ml Erlenmeyer flask. Evaporate ether on water bath and dry residue at 100° for 10 min. Add 2 ml of neutral alcohol and 20 ml of H<sub>2</sub>O and heat to dissolve acid. Cool, filter thru Gooch crucible, add drop or two of phenolphthalein indicator soln, and titrate with 0.02 N NaOH soln, of which 1 ml = 0.00374 g of Pyrethrin II.

## PYRETHRUM EXTRACTS IN MINERAL OIL

## PYRETHRIN I

*Mercury Reduction Method<sup>34</sup>—Tentative*

## 115

REAGENTS.—See 112

## 116

## DETERMINATION

Weigh or measure a quantity of sample that will contain 20–75 mg of Pyrethrin I, and transfer into 300 ml Erlenmeyer flask.

Add 20 ml or more if necessary of normal alcoholic NaOH soln to flask containing pyrethrum extract, connect to reflux condenser, and boil gently 1–1.5 hours. Transfer to 600 ml beaker and add sufficient H<sub>2</sub>O to make aqueous layer to 200 ml. If more than 20 ml of alcoholic soda has been used, add sufficient H<sub>2</sub>O so that all

alcohol will be removed when volume has been reduced to 150 ml. Add a few glass beads, or preferably use boiling tube, and boil aqueous layer down to 150 ml. Transfer contents of beaker to 500 ml separatory funnel and draw off aqueous layer into 250 ml volumetric flask. Wash oil layer once with  $H_2O$  and add wash  $H_2O$  to aqueous portion. After drawing off aqueous layer and washings, if slight emulsion still persists, it may be broken by addition of 2-3 ml of 10%  $BaCl_2$  soln. Do not shake vigorously after adding the  $BaCl_2$ , otherwise reversed emulsion that is difficult to separate may be formed. To aqueous soln in the 250 ml flask, add 1 g of filter-cel and 10 ml or more of the  $BaCl_2$  soln. Do not shake before making to volume. Make to volume, mix thoroly and filter off 200 ml. Test filtrate with  $BaCl_2$  to see if sufficient has been added to obtain clear soln. Neutralize with  $H_2SO_4$  (1+4) and add 1 ml in excess, using 1 drop of phenolphthalein as indicator. From this point, proceed as directed under 113, beginning "Filter thru 7 cm filter paper."

NOTE: Chrysanthemum monocarboxylic acid reacts with Denigès' reagent to form a series of colors beginning with phenolphthalein red, which gradually changes to purple, then blue, and finally to bluish green. The color reaction is very distinct with 5 mg of monocarboxylic acid and quantities as low as 1 mg can usually be detected. Therefore no Pyrethrin I should be reported if color reaction is negative.

When analyzing samples containing much perfume or other saponifiable ingredients such as thiocyanates, it may be necessary to use as much as 50 ml of normal alcoholic NaOH.

## FORMALDEHYDE SOLUTIONS

### FORMALDEHYDE

#### *Hydrogen Peroxide Method*<sup>3a</sup>—Official

117

#### REAGENTS

(a) *Sulfuric acid*.—1 N. Prepare as directed under II, 19(b).

(b) *Sodium hydroxide soln*.—1 N. Standardize against (a), using litmus or bromothymol blue indicator. 1 ml = 30.03 mg of HCHO.

(c) *Hydrogen peroxide soln*.—Commercial, containing ca 3% of  $H_2O_2$ . If acid, neutralize with the NaOH (b), using litmus or bromothymol blue indicator.

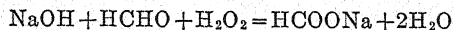
(d) *Litmus indicator*.—A soln of purified litmus of such concentration that 3 drops will impart a distinct blue color to 50 ml. of  $H_2O$ .

(e) *Bromothymol blue indicator*.—Dissolve 1 g of bromothymol blue in 500 ml of alcohol, 50% by volume.

118

#### DETERMINATION

Measure 50 ml of the NaOH soln into 500 ml Erlenmeyer flask and add 50 ml of the  $H_2O_2$ . Add weighed quantity, ca 3 g, of sample, allowing point of weighing pipet to reach nearly to liquid in flask. Place funnel in neck of flask and heat on steam bath 5 min., shaking occasionally. Remove from steam bath, wash funnel with  $H_2O$ , cool flask to room temp., and titrate excess NaOH with the 1 N acid, using the bromothymol blue or litmus indicator. (It is necessary to cool flask before titration with the acid to obtain a sharp end point with the litmus.) From amount of 1 N NaOH consumed and weight of sample calculate percentage of HCHO according to following equation:



If the HCHO soln contains an appreciable quantity of free acid, titrate a separate portion and calculate acidity as percentage of HCOOH. Make correction for this acidity in calculating percentage of HCHO.

119

*Cyanide Method*<sup>37</sup>—Official

(Applicable only to dilute solutions.)

Treat 15 ml of 0.1 *N* AgNO<sub>3</sub> soln, 89, with 6 drops of HNO<sub>3</sub> (1+1) in 50 ml volumetric flask, add 10 ml of KCN soln (3.1 g KCN dissolved in 500 ml of H<sub>2</sub>O), dilute to mark, shake well, filter thru dry filter, and titrate 25 ml of filtrate with 0.1 *N* NH<sub>4</sub>SCN, 91(a), as directed under XII, 37. Acidify another 15 ml portion of 0.1 *N* AgNO<sub>3</sub> with 6 drops of the dilute HNO<sub>3</sub> and treat with 10 ml of the KCN soln to which has been added a measured quantity (weight must be calculated from sp. gr.) of sample containing not over 25 mg of HCHO. Dilute to 50 ml, filter, and titrate 25 ml aliquot with the 0.1 *N* NH<sub>4</sub>SCN for excess of Ag as before. Difference between ml of NH<sub>4</sub>SCN used in these 2 titrations  $\times 2$  = ml of 0.1 *N* NH<sub>4</sub>SCN corresponding to KCN used by the HCHO. Calculate percentage of HCHO present. 1 ml of 0.1 *N* NH<sub>4</sub>SCN = 3 mg of HCHO.

## LIME-SULFUR SOLUTIONS

TOTAL SULFUR<sup>38</sup>—OFFICIAL

(Sulfur-free reagents should be used.)

120

## PREPARATION OF SAMPLE

Weigh ca 10 g of soln, transfer to 250 ml volumetric flask, and immediately dilute to mark with recently boiled and cooled H<sub>2</sub>O. Mix thoroly and transfer to number of small bottles, filling them completely and avoiding contact of soln with air as much as possible. Stopper bottles, seal with paraffin, and preserve in dark, cool place.

121

## DETERMINATION

Dissolve 2–3 g of Na<sub>2</sub>O<sub>2</sub> in 50 ml of cold H<sub>2</sub>O in 250 ml beaker. Transfer 10 ml aliquot of the prepared soln to this aqueous soln of Na<sub>2</sub>O<sub>2</sub>, keeping tip of pipet constantly just under surface of liquid until necessary to raise it for drainage at end. Use clean dry pipet for measuring each portion. Cover beaker with watch-glass and heat on steam bath, with occasional stirring, until all S is oxidized to sulfate (indicated by disappearance of yellow color). Wash off watch-glass and sides of beaker, acidify with HCl (1+4), evaporate to complete dryness, treat with H<sub>2</sub>O acidified with HCl, boil, and filter to remove SiO<sub>2</sub>. Dilute filtrate to 300 ml, add 50 ml of HCl, heat to boiling, and add 10% BaCl<sub>2</sub> soln (11 ml for 1 g of BaSO<sub>4</sub>) with constant stirring, at such rate that ca 4 min. is required for running in necessary quantity. (Rate may be regulated by attaching suitable capillary tip to buret containing the BaCl<sub>2</sub> soln.) Evaporate to dryness on steam bath, take up with hot H<sub>2</sub>O, filter thru quantitative filter, wash until free from chlorides, ignite carefully, and heat to *constant weight* over Bunsen burner. Calculate percentage of S from weight of BaSO<sub>4</sub>, using factor 0.1374.

MONOSULFIDE EQUIVALENT<sup>39</sup>—TENTATIVE

122

## REAGENT

*Iodine soln.*—0.1 *N*. Prepare as directed under 3(b), using 12.7 g of I and 25 g of KI.

123

## DETERMINATION

Dilute 10 ml of prepared soln, 120, to ca 30 ml with recently boiled and cooled H<sub>2</sub>O and titrate with the 0.1 *N* I soln until yellow color just disappears. (There



should be no difficulty in determining this end point; if there is, a small crystal of Na nitroprusside may be used, but it must not be added until end point is practically reached, because the blue color, if well developed, cannot be destroyed except by excess of I.) From number of ml of 0.1 *N* I soln used calculate percentage of monosulfide equivalent. 1 ml of 0.1 *N* I = 0.001603 g of S as monosulfide equivalent.

#### THIOSULFATE SULFUR

##### *Zinc Chloride Method*<sup>38</sup>—Official

124

#### REAGENT

*Ammoniacal zinc chloride soln.*—Dissolve 50 g of pure ZnCl<sub>2</sub> in ca 500 ml of H<sub>2</sub>O, add 125 ml of NH<sub>4</sub>OH and 50 g of NH<sub>4</sub>Cl, and dilute to 1 liter.

125

#### DETERMINATION

To 50 ml of H<sub>2</sub>O in 200 ml volumetric flask, add in manner indicated under 121, 50 ml of soln prepared as directed under 120. Add slight excess of the ammoniacal ZnCl<sub>2</sub> soln and dilute to mark. Shake thoroly and filter thru dry filter. To 100 ml of filtrate add few drops of methyl orange or methyl red indicator, 3(f) or II, 19(i), and exactly neutralize with 0.1 *N* HCl. Titrate the neutral soln with 0.05 *N* I soln, 3(b), using few drops of starch indicator, 3(e). From number of ml of I soln used calculate percentage of thiosulfate S present. As value of the I soln is given in terms of As<sub>2</sub>O<sub>3</sub>, multiply this value by 1.296 to obtain equivalent of thiosulfate S.

126

##### *Iodine Titration Method*<sup>39</sup>—Tentative

Continue titration of soln used in determination of the monosulfide equivalent, 123, with the 0.1 *N* I soln, letting the I act as its own indicator until a small drop produces a slight permanent coloration. From number of ml of 0.1 *N* I used calculate percentage of thiosulfate S. 1 ml of 0.1 *N* I = 0.006412 g of S as thiosulfate.

#### SULFIDE SULFUR

127

##### *Zinc Chloride Method*<sup>38</sup>—Official

To 10–15 ml of H<sub>2</sub>O in small beaker, add in manner indicated under 121, 10 ml aliquot of the soln prepared as directed under 120. Calculate quantity of ammoniacal ZnCl<sub>2</sub> soln, 124, necessary to precipitate all the S in the aliquot and add slight excess. Stir thoroly, filter, wash precipitate twice with cold H<sub>2</sub>O, and transfer filter paper and precipitate to beaker in which precipitation was made. Cover with H<sub>2</sub>O, disintegrate paper with glass rod, and add ca 3 g of Na<sub>2</sub>O<sub>2</sub>, keeping beaker well covered with watch-glass. Warm on steam bath with frequent shaking until all S is oxidized to sulfate, adding more Na<sub>2</sub>O<sub>2</sub> if necessary. Make slightly acid with HCl (1+4), filter to remove shreds of filter paper, wash thoroly with hot H<sub>2</sub>O, and determine S in filtrate as directed under 121.

128

##### *Iodine Titration Method*<sup>39</sup>—Tentative

Allow soln from 126 to stand several hours with occasional stirring, or acidify with few drops of HCl (1+4); warm gently with stirring, filter, and wash thoroly with warm H<sub>2</sub>O. Place filter paper with S in a small vessel and dissolve S in ca 15 ml of NaOH soln, 3(d), by heating gently on steam or water bath 1–1.5 hours (do not boil). Keep flask covered and shake gently few times during digestion to remove S from sides. Oxidize by adding 2–3 g of Na<sub>2</sub>O<sub>2</sub> dissolved in 50 ml of cold H<sub>2</sub>O and

complete determination as directed under 121, beginning "Cover beaker with watch-glass."

129

*Indirect Method—Tentative*

The difference between total S and the sum of thiosulfate S and sulfate S is sulfide S.

**SULFATE SULFUR**

130

*Zinc Chloride Method—Official*

Slightly acidify soln from determination of thiosulfate S, 125, with HCl (1+4), heat to boiling, add slowly and with constant stirring slight excess of 10% BaCl<sub>2</sub> soln, boil 30 min., allow to stand overnight, and filter. Calculate S from weight of BaSO<sub>4</sub>, and report as percentage of sulfate S.

131

*Iodine Titration Method<sup>39</sup>—Tentative*

To filtrate from determination of thiosulfate S, 126, add several drops of HCl, precipitate in cold with 5 ml of 10% BaCl<sub>2</sub> soln, allow to stand overnight, and filter. Calculate S from weight of BaSO<sub>4</sub>, and report as percentage of sulfate S.

132

**TOTAL LIME<sup>38</sup>—OFFICIAL**

To 25 ml of prepared soln, 120, add 10 ml of HCl, evaporate to dryness on steam bath, treat with H<sub>2</sub>O and few ml of HCl (1+4), warm until all CaCl<sub>2</sub> is dissolved, and filter to remove S and any SiO<sub>2</sub> that may be present. Dilute filtrate to volume of 200–250 ml, heat to boiling, and add few ml of NH<sub>4</sub>OH in excess, and then excess of saturated soln of (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub>. Continue boiling until precipitated CaC<sub>2</sub>O<sub>4</sub> assumes well defined granular form, allow to stand an hour, filter, and wash few times with hot H<sub>2</sub>O. Ignite in a Pt crucible over blast lamp to constant weight and calculate to percentage of CaO.

**ORGANIC MERCURIAL SEED DISINFECTANTS****MERCURY***Volatilization Method<sup>40</sup>—Official*

133

**APPARATUS**

The apparatus (Fig. 10) consists of 2 flanged crucibles that can be clamped mouth to mouth by means of 2 rings and screws. The lower crucible is made of Fe and the upper one of Au. The opening of the Au crucible is slightly larger than that of the other, so that there will be no tendency for the Hg to lodge in the joint between the two flanges. The Au crucible is fitted with a cooling device by which H<sub>2</sub>O may be slowly circulated thru large tube attached to it by Gooch tubing. The assembled apparatus rests on an asbestos board having a hole just large enough to receive crucible.

134

**DETERMINATION**

Weigh 1 g of sample into the Fe crucible and mix thoroly with 5 g of anhydrous Na<sub>2</sub>CO<sub>3</sub>. Cover mixture with thin layer of Na<sub>2</sub>CO<sub>3</sub> and then with 10 g of finely powdered BaCO<sub>3</sub>. Put weighed Au crucible in place, clamp the two together, set Fe crucible in place in asbestos board, start cooling H<sub>2</sub>O, and gently heat Fe crucible. Do not run H<sub>2</sub>O too fast because Hg amalgamates best with Au crucible if temp. is allowed to rise to ca 50°. Heat below red heat 30 min., cool, remove Au

crucible, wash it with alcohol, dry with heat of hand, and place in  $\text{CaCl}_2$  desiccator until it attains constant weight. Calculate increase in weight of Au crucible as percentage of metallic Hg in sample. If product contains more than 12% Hg, use less than 1 g because the Au crucible can safely retain ca 0.12 g of Hg. Re-

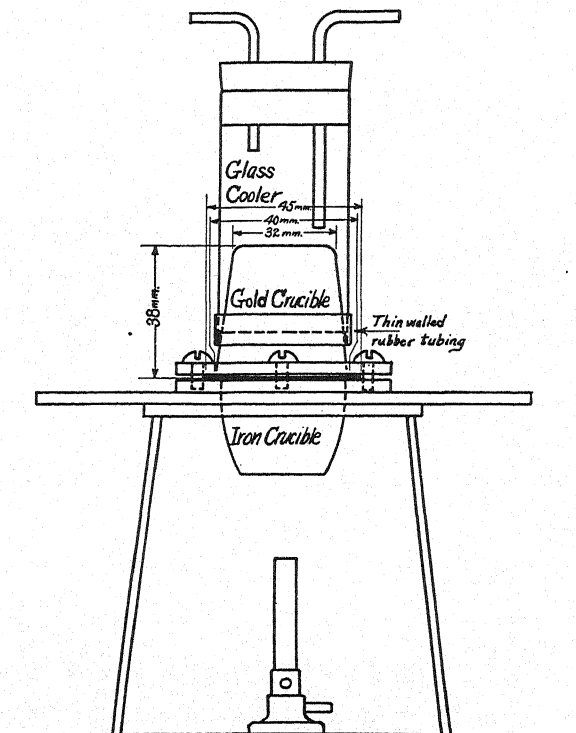


FIG. 10.—APPARATUS USED IN VOLATILIZATION METHOD FOR DETERMINATION OF MERCURY

move Hg from the Au crucible, preparatory to another experiment, by short ignition at dull red heat under hood having good draught. (The crucible will melt in the full heat of Bunsen burner.)

#### *Precipitation Method<sup>40</sup>—Official*

135

#### REAGENT

*Hydrogen peroxide soln.*—30%. Commonly designated as “perhydrol” or “super-oxol.”

136

#### DETERMINATION

Place 0.5–2.0 g of sample, depending on quantity of Hg present, in 200 ml Erlenmeyer flask fitted with air condenser by means of ground-glass joint. Add 10 ml of  $\text{H}_2\text{SO}_4$ , connect flask to condenser, and rotate in order to bring all the sample into contact with the acid. Add dropwise thru condenser tube 3–5 ml of the  $\text{H}_2\text{O}_2$  soln, and mix by rotation of flask. After active reaction has subsided, heat over low flame 15–20 min., add 5 ml more of the  $\text{H}_2\text{O}_2$ , and continue heating until all organic matter is destroyed (indicated by a clear soln), adding more  $\text{H}_2\text{O}_2$  if necessary. Remove

flask from heat, allow to cool, wash down condenser, and transfer contents to beaker, filtering if necessary. Dilute to ca 200 ml and destroy excess of  $\text{H}_2\text{O}_2$  by titration with  $\text{KMnO}_4$  soln. Precipitate the Hg with  $\text{H}_2\text{S}$ , filter thru weighed Gooch crucible, and dry precipitate in oven at  $105\text{--}110^\circ$ . Extract dried precipitate with  $\text{CS}_2$  to remove any precipitated S, again dry, and weigh. From weight of  $\text{HgS}$  calculate percentage of metallic Hg, using factor 0.86219.

### SODIUM HYPOCHLORITE SOLUTIONS<sup>41</sup>

#### SODIUM HYPOCHLORITE—OFFICIAL

##### *Arsenious Oxide Titration Method*

137

#### REAGENTS

(a) *Arsenious oxide soln.*—0.1 N. Dissolve exactly 2.474 g of pure  $\text{As}_2\text{O}_3$  in a beaker by boiling with 150–200 ml of  $\text{H}_2\text{O}$  containing 10 ml of  $\text{H}_2\text{SO}_4$ . Cool, add phenolphthalein indicator, neutralize acid with  $\text{NaOH}$  soln, adjust to faint acid reaction, transfer to a 500 ml volumetric flask, and dilute to mark.

(b) *Standard iodine soln.*—Prepare as directed under 3(b). Standardize against (a).

138

#### DETERMINATION

Transfer 20 ml aliquot of sample to liter volumetric flask and dilute to volume. Pipet 50 ml aliquot of mixture into 200 ml Erlenmeyer flask. Add the standard  $\text{As}_2\text{O}_3$  soln in excess and then add decided excess of  $\text{NaHCO}_3$ . Titrate excess of  $\text{As}_2\text{O}_3$  with the standard I soln, using starch soln, 3(e), or the I as indicator. Subtract volume of I soln, corrected to 0.1 N, from volume of  $\text{As}_2\text{O}_3$  soln used, and from this value and sp. gr. of soln, calculate percentage of  $\text{NaOCl}$ . 1 ml of 0.1 N  $\text{As}_2\text{O}_3$  soln = 0.003723 g of  $\text{NaOCl}$ .

139

#### AVAILABLE CHLORINE—OFFICIAL

Calculate percentage of available Cl from titration described under 138. 1 ml of the 0.1 N  $\text{As}_2\text{O}_3$  = 0.003546 g of available Cl.

140

#### CHLORIDE CHLORINE—OFFICIAL

Pipet 50 ml aliquot of prepared soln, 138, into 200 ml Erlenmeyer flask and add slight excess of the  $\text{As}_2\text{O}_3$  soln, 137(a), calculated from  $\text{NaOCl}$  titration; add slight excess of  $\text{HNO}_3$ , neutralize soln with  $\text{CaCO}_3$ , and titrate with 0.1 N  $\text{AgNO}_3$ , 89, using  $\text{K}_2\text{CrO}_4$ , II, 48(b), or the Na arsenate formed in the soln as indicator. Run blank determination on reagents and make correction for any Cl found. From this corrected titration and sp. gr. of sample calculate percentage of Cl. From this value subtract one-half the percentage of available Cl. Difference is percentage of chloride Cl.

141

#### SODIUM HYDROXIDE—OFFICIAL

Pipet 25 ml of sample into a 250 ml volumetric flask, and add sufficient  $\text{H}_2\text{O}_2$  soln (neutral to phenolphthalein) to destroy  $\text{NaOCl}$ . Mix well and add sufficient neutral 10%  $\text{BaCl}_2$  soln to precipitate carbonates, make to volume, mix thoroly, and filter thru dry filter. Pipet 50 ml of filtrate into Erlenmeyer flask and titrate with 0.1 N  $\text{HCl}$ , using phenolphthalein as indicator, 106(a). From this titration and sp. gr. of sample calculate percentage of  $\text{NaOH}$ .

## CARBON DIOXIDE—OFFICIAL

142

## APPARATUS

Connect an evolution flask, to which is attached a dropping funnel protected by a tube containing soda lime, to a condenser or Kjeldahl distilling trap, which in turn is connected to 2 wash bottles containing 10% KI soln. Use glass beads or other device in wash bottles to cause gas to flow slowly thru liquid. End train with Meyer absorption tube containing 0.1 N Ba(OH)<sub>2</sub> soln.

143

## DETERMINATION

Pipet suitable aliquot of sample (5–20 ml, governed by quantity of CO<sub>2</sub> present) into evolution flask, and attach flask to train. Place 50 ml of 0.1 N Ba(OH)<sub>2</sub> soln in the Meyer tube, and add 35–50 ml of H<sub>2</sub>O<sub>2</sub> soln (or sufficient quantity to reduce hypochlorite) thru dropping funnel into evolution flask. After action due to the H<sub>2</sub>O<sub>2</sub> has ceased, add 30 ml of HCl (1+3), heat flask to boiling, and draw air slowly thru apparatus. (Evolved gases will be freed from Cl by the KI in wash bottles, and the CO<sub>2</sub> will be absorbed in the standard Ba(OH)<sub>2</sub> in Meyer tube.) Draw air thru apparatus 20 min., disconnect Meyer tube, and pour its contents into beaker. Wash out tube, adding washings to contents of beaker. Filter, wash, and titrate filtrate and washings with 0.1 N HCl, using phenolphthalein as indicator, 106(a). From number of ml of Ba(OH)<sub>2</sub> used and sp. gr. of sample, calculate percentage of CO<sub>2</sub>. 1 ml of 0.1 N Ba(OH)<sub>2</sub> = 0.00220 g of CO<sub>2</sub>.

CALCIUM HYPOCHLORITE AND BLEACHING POWDER<sup>42</sup>

## AVAILABLE CHLORINE—OFFICIAL

144

*Arsenious Oxide Titration Method*

Weigh 5–10 g of thoroly mixed sample into porcelain mortar, add 30–40 ml of H<sub>2</sub>O, and triturate until smooth cream is obtained (high-test Ca(OCl)<sub>2</sub> will dissolve readily and not form a cream). Add more H<sub>2</sub>O, stir well with pestle, and allow insoluble residue to settle few moments. Pour mixture off into liter volumetric flask, add more H<sub>2</sub>O, and thoroly triturate sample and pour off as before. Repeat operation until all material has been transferred to flask. Rinse mortar and pestle, catch wash H<sub>2</sub>O in flask, dilute soln to mark, and mix. Without allowing material to settle, pipet 25–50 ml aliquot into 200 ml Erlenmeyer flask. Add the standard As<sub>2</sub>O<sub>3</sub> soln, 137(a), in excess and then add a decided excess of NaHCO<sub>3</sub>. Titrate excess of As<sub>2</sub>O<sub>3</sub> with the standard I soln, 3(b), using starch soln, 3(e), or the I as indicator. Subtract volume of I-soln, corrected to 0.1 N, from volume of As<sub>2</sub>O<sub>3</sub> soln used, and calculate percentage of available Cl. 1 ml of 0.1 N As<sub>2</sub>O<sub>3</sub> = 0.003546 g of available Cl.

CHLORAMINE-T<sup>42</sup>

## ACTIVE CHLORINE—OFFICIAL

145

*Arsenious Oxide Titration Method*

REAGENTS.—See 137.

146

## DETERMINATION

Transfer 0.5 g of sample to 300–500 ml Erlenmeyer flask, dissolve in 50 ml of H<sub>2</sub>O, and add excess of the standard As<sub>2</sub>O<sub>3</sub> soln, 137(a), and 5 ml of H<sub>2</sub>SO<sub>4</sub> (1+4). Add decided excess of NaHCO<sub>3</sub> and titrate excess As<sub>2</sub>O<sub>3</sub> with standard I soln, 3(b),

using starch soln, 3(e), or the I as indicator. From this titration calculate active Cl in sample. 1 ml of 0.1 N  $\text{As}_2\text{O}_3$  soln = 0.001773 g of active Cl.

147

## TOTAL CHLORINE—OFFICIAL

Dissolve 0.5 g of sample in 50 ml of  $\text{H}_2\text{O}$  in Erlenmeyer flask and add slight excess of the standard  $\text{As}_2\text{O}_3$  soln calculated from the active Cl titration, 146. Add 5 ml of  $\text{HNO}_3$  (1+4), neutralize with  $\text{CaCO}_3$ , and titrate with standard  $\text{AgNO}_3$ , 89, using  $\text{K}_2\text{CrO}_4$ , II, 48(b), as indicator. Run blank titration on reagents and make correction for any Cl found. From corrected titration calculate percentage of total Cl in sample. 1 ml of 0.1 N  $\text{AgNO}_3$  = 0.003546 g of Cl. If total Cl exceeds active Cl, presence of NaCl is indicated.

148

## SODIUM—OFFICIAL

Weigh 0.5 g of sample in silica or porcelain dish and add ca 25 ml of  $\text{H}_2\text{O}$  and 3–5 ml of  $\text{H}_2\text{SO}_4$  (1+4). Evaporate to sirupy consistency on steam bath and finally to dryness on hot plate. Ignite at full heat of Bunsen burner, cool, and weigh as  $\text{Na}_2\text{SO}_4$ . (Residue should be completely soluble in  $\text{H}_2\text{O}$  and should show no turbidity with  $\text{NH}_4\text{OH}$  and  $(\text{NH}_4)_2\text{CO}_3$ .) Test with flame for Na. If residue meets these tests it may be considered pure  $\text{Na}_2\text{SO}_4$ . From weight of residue calculate percentage of Na in sample.

PHENOL COEFFICIENT<sup>43</sup>—OFFICIALI. USING *EBERTHELLA TYPHOSA*

(Applicable to testing of coal tar disinfectants that are miscible with  $\text{H}_2\text{O}$  and to other disinfectants that are miscible with  $\text{H}_2\text{O}$  and act against bacteria in manner similar to phenol. False values are obtained from certain products that are highly inhibitory, such as Hg compounds, and the values obtained from testing oxidizing products may be highly misleading.)

149

## REAGENTS

(a) *Culture media*.—(1) *Nutrient broth*: Boil 5 g of Liebig's beef extract, 5 g of NaCl, and 10 g of Armour's peptone (quality specially prepared for disinfectant testing) in 1000 ml of  $\text{H}_2\text{O}$  20 min., make up to volume with  $\text{H}_2\text{O}$ , and adjust to pH 6.8 (using colorimetric method, adjust broth to give dark green color with bromothymol blue). Filter thru paper, place 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs pressure 40 min. Use this broth for daily transfers and for subcultures. (2) *Nutrient agar*: Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to pH 7.2–7.4 (which gives a blue-green color with bromothymol blue), tube, plug with cotton, sterilize, and slant.

(b) *Test organism*.—The Hopkins strain of *Eberthella typhosa* (Zopf) Weldin (frequently called *Bac. typhosus*). Carry a stock culture on nutrient agar slants. Transfer once a month and incubate new stock transfer 2 days at 37°, then store at room temp.

From the stock culture inoculate a tube of nutrient broth and make at least 4 consecutive daily transfers (not over 30) in nutrient broth, incubating at 37°, before using culture for testing (if only 1 daily transfer has been missed it is not necessary to repeat the 4 consecutive transfers). Use 22–26 hours' culture of organism grown in nutrient broth at 37° in test. Shake, and allow to settle 15 min. before using.

(c) *Phenol*.<sup>44</sup>—Use phenol that meets requirements of U.S.P. and has congealing point 40° or above. Use 5% soln as stock soln and keep in well-stoppered amber

bottles in relatively cool place, protected from light. Standardize with 0.1 N bromine or Na bromide-bromate soln.<sup>45</sup>

## 150

## APPARATUS

(a) *Glassware*.—1, 5, and 10 ml volumetric pipets; 1, 5, and 10 ml Mohr pipets graduated to 0.1 ml or less; 100 ml stoppered cylinders graduated in 1 ml divisions. Pyrex lipped test tubes 25 × 150 mm. Plug test tubes (medication tubes) with cotton wrapped in 1 layer of cheese-cloth. Sterilize all glassware 2 hours in hot air oven at 180°. Place pipets in closed metal containers before sterilizing.

(b) *Water bath*.—An insulated relatively deep water bath with cover having at least 10 well-spaced holes which admit medication tubes but not their lips.

(c) *Racks*.—May be of any convenient style. Blocks of wood (size depending somewhat on incubator to accommodate them) with deep holes are satisfactory. Have holes well-spaced to insure quick manipulation of tubes; it is convenient to have them large enough to admit medication tubes while dilutions are being made.

(d) *Transfer loop*.—Make 4 mm (inside diameter) single loop at end of 2–3" Pt wire No. 23 B & S Gage. Have other end in suitable holder (glass or Al rod). Bend loop at a 30° angle with stem (Fig. 11).

## 151

## PROCEDURE

Make 1% stock dilution of substance to be tested (or any other convenient dilution, depending on anticipated strength) in glass-stoppered cylinder. Make final dilutions, from the 1% stock dilution, directly into medication tubes and remove all excess over 5 ml. (Range of dilutions should cover killing limits of the disinfectant within 5 and 15 min. periods and should at the same time be sufficiently close for accuracy.) From the 5% stock soln make a 1–90 and a 1–100 dilution of the phenol directly into medication tubes. Place these tubes, containing 5 ml each of final dilutions of disinfectant and of phenol, in water bath at 20° and leave 5 min. Add 0.5 ml of the test culture to each of the dilutions at time intervals corresponding to intervals at which transfers are to be made. (Thus, by the time 10 tubes have been seeded at 30 second intervals, 4.5 min. will have elapsed, and a 30 second interval intervenes before transference to sub-cultures is commenced.) Add culture from graduated pipet of sufficient capacity to seed all tubes in any one set. (As a precautionary measure the pipet should be loosely plugged with cotton at mouth end before being sterilized. Temp. of culture should be practically that of water bath before it is added.)

In inoculating medication tubes, hold them in slanting position after removal from bath, insert pipet to just above surface of disinfectant, and run in culture without allowing tip to touch disinfectant. After adding culture, agitate tubes gently but thoroly to insure even distribution of bacteria, and replace in bath; 5 min. after seeding first medication tube, transfer 1 loopful of mixture of culture and diluted disinfectant from medication tube to corresponding sub-culture tube. To facilitate transfer of uniform drops of medication mixture, hold tube at 60° angle, and withdraw loop so that plane of loop is parallel with surface of liquid (see Fig. 11). At end of 30 seconds, transfer loopful from second medication tube to second sub-culture tube and continue process for each successive dilution; 5 min. after making first transfer begin second set of transfers for 10 min. period, and finally repeat for 15 min. period. Before each transfer heat loop to redness in Bunsen flame and flame mouth of every tube. Sterilize loop immediately after each transfer (before replugging tubes) to allow time for cooling. Use care in transferring and seeding to prevent pipet or needle from touching sides or mouth of medication tube and see

that no cotton threads adhere to inner sides or mouths of tubes. Incubate subcultures at 37° for 48 hours and read results. Macroscopic examination is usually sufficient. Occasionally a 3-day incubation period, an agar streak, a microscopical examination, or agglutination with antityphoid serum may be necessary to determine feeble growth or suspected contamination.

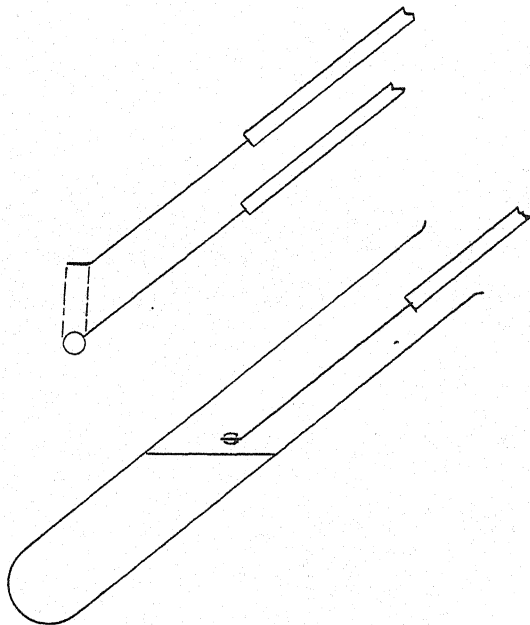


FIG. 11.—TRANSFER LOOP AND MANNER OF USING IN DETERMINATION OF PHENOL COEFFICIENT

## 152

## CALCULATION

Express results in terms of phenol coefficient, a number obtained by dividing numerical value of greatest dilution (denominator of fraction expressing dilution) of the disinfectant capable of killing *Eb. typhosa* in 10 min. but not in 5 min. by greatest dilution of phenol showing same results.

Example:

	5 MIN.	10 MIN.	15 MIN.
Disinfectant (X):			
1-300	0	0	0
1-325	+	0	0
1-350	+	0	0
1-375	+	+	0
1-400	+	+	+
Phenol:			
1-90	+	0	0
1-100	+	+	+

Phenol coefficient would be  $\frac{350}{90} = 3.89$ .

The test is satisfactory only when phenol control gives one of following readings:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1-90	+ or 0	+ or 0	0
1-100	+	+	+ or 0



If none of dilutions of disinfectant shows growth in 5 min. and killing in 10 min., estimate hypothetical dilution only when any 3 consecutive dilutions show following results: The first, no growth in 5 min.; the second, growth in 5 and 10 min. but not in 15 min.; and the third, growth in 5, 10, and 15 min.

Example:

	5 MIN.	10 MIN.	15 MIN.
Disinfectant (X):			
1-300	0	0	0
1-350	+	+	0
1-400	+	+	+
Phenol:			
1-90	0	0	0
1-100	+	+	0

Phenol coefficient would be  $\frac{325}{95} = 3.42$ .

To avoid giving an impression of fictitious accuracy, calculate phenol coefficient to nearest 0.1. Thus, in examples cited above, phenol coefficients would be reported as 3.9 and 3.4, instead of 3.89 and 3.42.

NOTE.—The commonly accepted criterion that disinfectants for general use be employed at a dilution equivalent in germicidal efficiency to 5% phenol against *Eb. typhosa* (that is, 20 times the *Eb. typhosa* coefficient) allows reasonable margin of safety for destruction of infective agents likely to be the object of general disinfection.

## 153

### II. USING STAPHYLOCOCCUS AUREUS<sup>43</sup>

(Applicable in bacteriological examination of disinfectants to be used for special purposes, such as disinfection of dental and surgical or veterinary instruments.)

Proceed as directed in 149-152, except to change the phenol dilutions. Use temp. of 20° unless otherwise directed. The culture of *Staph. aureus* must have at least the resistance indicated by the following:

AT 20°	PHENOL	5 MIN.	10 MIN.	15 MIN.
	1-60	+	0	0
	1-70	+	+	+

The resistance of the culture to phenol when used at 37° must be as follows:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1-80	+	0	0
1-90	+	+	+ or 0

### SELECTED REFERENCES

- <sup>1</sup> J. Ind. Eng. Chem., 8, 327 (1916); J. Assoc. Official Agr. Chem., 3, 158 (1917).
- <sup>2</sup> Z. anal. Chem., 32, 415 (1893); J. prakt. Chem., 91, 133 (1915); J. Assoc. Official Agr. Chem., 5, 33 (1921).
- <sup>3</sup> J. Ind. Eng. Chem., 14, 207 (1922); J. Assoc. Official Agr. Chem., 5, 402 (1922); 6, 313 (1923).
- <sup>4</sup> J. Assoc. Official Agr. Chem., 7, 313 (1924).
- <sup>5</sup> Ibid., 5, 398 (1922).
- <sup>6</sup> J. Am. Chem. Soc., 40, 1036 (1918); J. Assoc. Official Agr. Chem., 5, 398 (1922).
- <sup>7</sup> Bur. Standards J. Research, 3, 581 (1929).
- <sup>8</sup> Compt. rend., 173, 714, 836 (1921); J. Assoc. Official Agr. Chem., 14, 253 (1931).
- <sup>9</sup> J. Am. Chem. Soc., 55, 1741 (1933); Ind. Eng. Chem., Anal. Ed., 5, 7 (1933); 9, 551 (1937); 11, 21 (1939); J. Assoc. Official Agr. Chem., 21, 459 (1938).
- <sup>10</sup> J. Ind. Eng. Chem., 1, 208 (1909); J. Assoc. Official Agr. Chem., 3, 158 (1917).
- <sup>11</sup> J. Assoc. Official Agr. Chem., 5, 34 (1921).
- <sup>12</sup> J. Am. Chem. Soc., 24, 1082 (1902).

- <sup>13</sup> U. S. Dept. Agr. Bur. Chem. Bull. 105, p. 167.
- <sup>14</sup> J. Assoc. Official Agr. Chem., 3, 332 (1920).
- <sup>15</sup> Ibid., 333.
- <sup>16</sup> U. S. Dept. Agr. Bur. Chem. Bull. 137, p. 40; 152, p. 68.
- <sup>17</sup> Ibid., 105, p. 166.
- <sup>18</sup> J. Assoc. Official Agr. Chem., 5, 33, 392 (1922).
- <sup>19</sup> Fresenius, Quantitative Chemical Analysis. Trans. 6th German ed., 1906, amplified and revised, Vol. 2, 1180; U. S. Geol. Survey Bull., 700, p. 218.
- <sup>20</sup> J. Assoc. Official Agr. Chem., 15, 289 (1932); 17, 63 (1934).
- <sup>21</sup> Ibid., 10, 27 (1927).
- <sup>22</sup> Ibid., 28.
- <sup>23</sup> Ibid., 29.
- <sup>24</sup> Ibid., 9, 27 (1926).
- <sup>25</sup> J. Ind. Eng. Chem., 12, 486 (1920).
- <sup>26</sup> U. S. Dept. Agr. Bur. Chem. Circ. 10, p. 5.
- <sup>27</sup> J. Assoc. Official Agr. Chem., 10, 30, 124 (1927); 11, 35 (1928).
- <sup>28</sup> Ibid., 9, 28 (1926).
- <sup>29</sup> Ibid.; U. S. Dept. Agr. Bur. Chem. Bull. 105, p. 165.
- <sup>30</sup> J. Assoc. Official Agr. Chem., 9, 29 (1926).
- <sup>31</sup> Sutton. Systematic Handbook of Volumetric Analysis, 10th ed., 1911, p. 61.
- <sup>32</sup> U. S. Dept. Agr. Bur. Animal Ind. Bull. 133.
- <sup>33</sup> Ind. Eng. Chem., Anal. Ed., 10, 19 (1938); J. Assoc. Official Agr. Chem., 21, 148 (1938); 22, 408 (1939).
- <sup>34</sup> Contrib. Boyce Thompson Inst., 8, No. 3, 175 (1936); Ind. Eng. Chem., Anal. Ed., 10, 5 (1938).
- <sup>35</sup> Soap, 10, No. 5, 89 (1934).
- <sup>36</sup> Ber., 31, 2979 (1898); J. Am. Chem. Soc., 27, 1183 (1905); U. S. Dept. Agr. Bur. Chem. Bull. 99, p. 30; 132, p. 49; 137, p. 47.
- <sup>37</sup> Z. anal. Chem., 36, 18 (1897); U. S. Dept. Agr. Bur. Chem. Bull. 132, p. 49.
- <sup>38</sup> J. Assoc. Official Agr. Chem., 3, 353 (1920).
- <sup>39</sup> Ibid., 4, 146 (1920).
- <sup>40</sup> Whitmore, Organic Compounds of Mercury, The Chemical Catalog Co., p. 365; J. Assoc. Official Agr. Chem., 13, 156 (1930); 15, 67 (1932).
- <sup>41</sup> J. Assoc. Official Agr. Chem., 18, 63 (1935).
- <sup>42</sup> Ibid., 65.
- <sup>43</sup> U. S. Dept. Agr. Circ. 198 (1931).
- <sup>44</sup> Sutton, Systematic Handbook of Volumetric Analysis, p. 404. P. Blakistons Sons & Co. (1924).
- <sup>45</sup> U. S. P. X, p. 283.

## VII. CAUSTIC POISONS

### PHENOL

#### *Method I—Official*

(Applicable to determination of phenol in commercial cresols, saponified cresol solns, coal tar dips, and disinfectants, and to kerosene solns of phenols except in presence of salicylates or betanaphthol.)

#### 1

##### REAGENTS

(a) *Dilute nitric acid*.—Blow air thru  $\text{HNO}_3$  until it is colorless and dilute 1 volume of this acid with 4 volumes of  $\text{H}_2\text{O}$ .

(b) *Millon's reagent*.—Treat 2 ml of Hg in 200 ml Erlenmeyer flask with 20 ml of  $\text{HNO}_3$ . Place flask under hood, and after first violent reaction is over shake as much as necessary to effect subdivision of Hg and maintain action. After ca 10 min., when action has practically ceased even in presence of undissolved Hg, add 35 ml of  $\text{H}_2\text{O}$ , and if basic salt separates, add sufficient quantity of the dilute  $\text{HNO}_3$  to dissolve it. Add 10% soln of NaOH dropwise with thoro mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity. Add 5 ml of the dilute  $\text{HNO}_3$  and mix well. As the soln deteriorates, do not use it after the first day.

(c) *Standard phenol soln*.—Dissolve weighed quantity of the pure substance (congealing point not lower than  $40^\circ$ ) in sufficient  $\text{H}_2\text{O}$  to make not less than 1% soln. On day it is to be used, from this stock soln make a 0.025% soln (final standard) in additional  $\text{H}_2\text{O}$ .

(d) *Formaldehyde soln*.—Dilute 2 ml of commercial 37%  $\text{HCHO}$  soln to 100 ml with  $\text{H}_2\text{O}$ .

#### 2

##### APPARATUS

(a) *Nessler cylinders*.—50 ml tall form, matched.

(b) *Test tubes*.—Approximately 180 mm  $\times$  20 mm, provided with rubber stoppers and marked at 25 ml.

(c) *Water bath for heating test tubes*.—A beaker containing disk of wire gauze raised about an inch from bottom may be used.

#### 3

##### PREPARATION OF SAMPLE

(a) *Commercial cresol*.—Weigh by difference ca 2.5 g of sample into 250 ml volumetric flask, dissolve in 10 ml of 10% NaOH soln, and make to mark with  $\text{H}_2\text{O}$ .

(b) *Saponified cresol solns, coal tar dips and disinfectants, kerosene solns of phenols, etc.*—Weigh by difference ca 5 g (or use 5 ml and calculate weight from density) of sample into 250 ml volumetric flask and dilute to mark with  $\text{H}_2\text{O}$ . In products consisting largely of kerosene, bring  $\text{H}_2\text{O}$  level to mark and take aliquots from aqueous portion only.

#### 4

##### DETERMINATION

Transfer 5 ml aliquot of prepared soln to 200 ml volumetric flask shortly before determination is to be carried out, dilute to ca 50 ml, add 1 drop of methyl orange indicator, VI, 3(f), and then the dilute  $\text{HNO}_3$  until soln is practically neutral, make to volume, and shake well.

Place 5 ml of the diluted soln in each of 2 of the marked test tubes, and in each of

2 additional test tubes place 5 ml of the standard phenol soln. Next flow 5 ml of the Millon's reagent down side of each tube, mix, and place tubes in bath of boiling  $H_2O$ ; continue boiling exactly 30 min.; cool immediately and thoroly by immersion in bath of cold  $H_2O$  at least 10 min., and add 5 ml of the dilute  $HNO_3$  to each tube.

Mix well and add 3 ml of the dilute  $HCHO$  soln to one of each pair of tubes; make all tubes to 25 ml mark with  $H_2O$ , stopper, shake well, and allow to stand overnight. The next day the contents of tubes to which  $HCHO$  was added will have faded to yellow, while the others will show orange or red tint.

Pipet 20 ml from each of the 2 phenol tubes and transfer to 100 ml volumetric flasks; treat each with 5 ml of the dilute  $HNO_3$ , make to mark, and mix. The red flask contains the "phenol standard," and the yellow flask the "phenol blank." Transfer these solns to burets. Pipet 10 ml of each sample soln into Nessler tubes. (The orange or red constitutes the "unknown" and the yellow the "sample blank," and each Nessler tube must be distinctly marked to avoid confusion.) Add to "sample blank" tube measured quantity of "phenol standard" and add same volume of "phenol blank" to "unknown," thoroly agitate (aided by insertion of rubber stoppers if necessary), and compare colors. When tubes have been brought to match, each ml of phenol standard used = 1% of phenol if portion of sample weighing exactly 5 g was used, or 2% if exactly 2.5 g was used.

NOTE.—In using this method the following precautions should be borne in mind: A pair of phenol tubes affords sufficient final solns for assaying several unknowns, but all the latter must have accompanied phenol solns thruout entire process with identical reagents and treatment. If end point has been inadvertently overrun it is possible to work back to it, but since mistakes are easy to make in this procedure it is better to repeat comparison on fresh portions from original tubes. Too much delay in matching tubes must be avoided after titration has been started, otherwise excess of  $HCHO$  present in blanks may have time after mixture to affect intensity of red color.

Millon's reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used.

5

#### *Method II<sup>2</sup>—Official*

(Applicable to determination of phenol in presence of salicylates.)

Weigh by difference into a separatory funnel 10 g of sample (or use 10 ml and calculate weight from density of sample). Add 50 ml of kerosene and extract 3 times with 100 ml portions of  $H_2O$ . Filter aqueous extracts thru wet filter into 500 ml volumetric flask, make to volume with  $H_2O$ , and proceed as directed under 4.

When tubes have been brought to match, each ml of the phenol standard used = 1% of phenol if a portion of sample weighing exactly 10 g was used.

#### SELECTED REFERENCES

<sup>1</sup> U. S. Dept. Agr. Bull., 1308, p. 17; J. Assoc. Official Agr. Chem., 13, 49 (1930).

<sup>2</sup> Ind. Eng. Chem., Anal. Ed., 1, 232 (1929).

## VIII. NAVAL STORES

### ROSIN

#### ANALYTICAL METHODS

1

##### SAMPLING—OFFICIAL, FIRST ACTION

Remove top 4" of the rosin in the barrel or drum, and by means of a sharp spike take single lump of ca 1 lb. from below original surface of rosin in barrel or drum. Do not break up or pulverize this lump. When acid number, saponification number, unsaponifiable matter, petroleum benzin insoluble or ash is to be determined obtain stated quantity by breaking off small pieces having freshly exposed surfaces, avoiding powdering as much as possible. (These precautions are necessary because rosin oxidizes rapidly on surface when exposed to air.)

2

##### ACID NUMBER—OFFICIAL, FIRST ACTION

To 2 g of the rosin in a 300 ml Erlenmeyer flask, add 50 ml of neutral 95% ethyl alcohol. (Denatured, Bur. Int. Rev. Formula No. 30 is suitable—1 vol. methyl to 10 vols. 95% alcohol.) Allow rosin to dissolve in alcohol at normal temp., or with aid of heat. Titrate with 0.5 N NaOH soln, using phenolphthalein, II, 10(d), as indicator. If necessary, in order to obtain sharp end point, dilute solns of the redder rosins with additional neutral alcohol. Calculate as acid number the mg of KOH required to neutralize 1 g of the rosin.

3

##### ACID NUMBER OF DARK COLORED ROSIN—TENTATIVE

With low-grade dark-colored rosin, where color of soln interferes with observation of end point, use following method based on use of a small direct vision hand spectroscope to observe appearance of an absorption band in green part of spectrum on liberation of the alkaline phenolphthalein color body:<sup>4</sup>

Place 100 ml of alcohol and 1 ml of phenolphthalein indicator soln in 300 ml Erlenmeyer flask. Add 0.5 N NaOH (1 or 2 drops should suffice) until an absorption band appears when viewing spectrum thru a 1" (2.5 cm) depth of liquid. Introduce 5 g of the rosin, previously weighed, into flask, stopper, and allow to dissolve at room temp. Titrate with the 0.5 N NaOH, running in ca 1 ml less than expected or theoretical quantity required for weight of sample taken. After further addition of each 0.05 or 0.10 ml of alkali, hold flask in inclined position towards a source of light, preferably daylight, and observe spectrum thru depth of ca 1". The end point is reached when an absorption band similar to that previously observed again becomes just perceptible.

4

##### SAPONIFICATION NUMBER—TENTATIVE

Weigh accurately 2 g of rosin sample into 250 ml Erlenmeyer flask and bring into soln in 25 ml neutral 95% alcohol (Formula No. 30 is suitable, 2). Add 20 ml of alcoholic KOH soln, XXXI, 24, allowing pipet to drain for definite time. Connect flask to reflux condenser (air condenser tube, 5 mm I.D.  $\times$  32" long will suffice), bring to boil on steam bath or hot plate, and hold at that temp. exactly 1 hour. Cool to room temp. and titrate with 0.5 N HCl, II, 19(a), using phenolphthalein as indicator. Dilute soln if necessary with neutral alcohol to obtain sharp end point. If dark color of soln prevents observation of end point, use spectroscopic method, 3. Disappearance from spectrum of characteristic absorption band then

marks end point, which is reached when addition of 1 or 2 drops of the acid causes disappearance of and addition of similar quantity of alkali brings back the band. Conduct several blank determinations, using same pipet for measuring the KOH soln and drain for same length of time. Ml of the 0.5 *N* HCl obtained in determination on sample subtracted from ml obtained on blank = ml of 0.5 *N* HCl equivalent to the KOH used in saponification of sample taken. Calculate as saponification number the mg of KOH required to saponify 1 g of rosin.

#### TOLUENE-INSOLUBLE MATERIAL<sup>2</sup>—OFFICIAL, FIRST ACTION

5

##### PREPARATION OF SAMPLE

(1) If sample is less than 200 g, immediately before making determination powder it to pass standard 10-mesh sieve, mix thoroly, and place in wide-mouthed bottle of such size that sample completely fills it.

(2) If sample is more than 200 g, crush it to pass a  $\frac{1}{2}$ " sieve, mix, quarter down to ca 200 g, and treat as directed in (1).

6

##### PROCEDURE

Place 50 g of freshly-powdered sample in 300 ml beaker, add 150 ml of toluene (free from H<sub>2</sub>O and non-volatile residue), and dissolve sample with aid of heat and occasional shaking. When soln is apparently complete (no particles of rosin visible), filter at once thru 25 ml porcelain Gooch crucible that has been previously prepared with mat of pure, well-washed asbestos and been finally washed thoroly with the solvent used, dried at 105–110° for 30 min., cooled in desiccator, and weighed. If rosin filtrate is not clear, return thru Gooch crucible until it is clear, finally washing residue and outside of crucible free from rosin with additional hot solvent. Dry crucible and contents to constant weight at 105–110° in oven (1 hour usually suffices), cool in desiccator, weigh, and calculate percentage of toluene insoluble.

7

##### PETROLEUM BENZIN-INSOLUBLE MATTER (OXIDIZED ROSIN)—TENTATIVE

Weigh 1 g of freshly pulverized rosin into tared 250 ml glass-stoppered (cork covered with tin foil may be used) Erlenmeyer flask. Add 100 ml of petroleum benzin (b.p. 30–75°), stopper flask, and shake to prevent rosin coalescing or adhering to flask. Add an additional 50 ml of petroleum benzin, stopper, and shake vigorously ca 5 min., or until any undissolved rosin is in finely divided state and does not adhere to flask. Allow flask to stand overnight at 23–28°, and filter soln thru tared Gooch crucible, rinsing flask with ca 50 ml of petroleum benzin. Wipe outside of flask and crucible with cloth wet with alcohol or acetone and dry in oven at 95–100° 1.5 hours. Cool, and weigh. From combined weights of residue in flask and in crucible calculate percentage of petroleum benzin-insoluble matter.

This method determines degree of oxidation. If rosin is appreciably "dirty," determine extraneous matter (toluene-insoluble matter) according to 5 and 6, and subtract quantity found from total quantity of petroleum ether-insoluble.

8

##### ASH—TENTATIVE

Weigh 10 g of rosin into porcelain crucible, burn off combustible matter slowly, and ignite residue until ash is free from carbonaceous matter. Cool crucible in desiccator and weigh. Report as percentage of ash.

9

##### VOLATILE OILS—TENTATIVE

Place 100 or 200 g of freshly broken lumps of rosin in liter distilling flask, set flask in oil bath maintained at 160–170°, and distil with steam, receiving distillate in

graduated cylinder. Measure exactly volume of separated oil and report as ml per 100 g of rosin.

### ROSIN GRADING

#### 10

##### SAMPLING—OFFICIAL, FIRST ACTION

Take sample by any of following procedures:

(a) Remove by spiking with a pointed heavy iron bar, (1) Fig. 12, a lump of rosin roughly 4–6" in diameter (2) from 6–8" below *the surface of the rosin* in the barrel or drum. With special rosin adz (3) or sampling hatchet (4) cut cube with parallel sides exactly  $\frac{7}{8}$ " apart (5).

(b) Immediately after barrel is filled, suspend in rosin in horizontal position a tubular mold  $\frac{7}{8}$ " square (inside) (6),  $1\frac{1}{2}$ " or more in length, made of thin, well-tinned plate. Place mold sufficiently deep (at least 6" in hot rosin) to insure depth of at least 4" below surface of rosin after it solidifies. After rosin has thoroly cooled, remove sample (7) by spiking as directed in (a).

(c) Remove in manner devised for the particular type of sampler (8) the sample (9) contained in a mold (8) made of well-tinned plate (which mold was placed in barrel or drum before it was filled with hot rosin) thru opening, the top of which is 8" from top of barrel or drum. Mold thus placed must be entirely within barrel or drum and completely encased in the rosin.

If any sample as prepared for grading is too large, so that it cannot be viewed thru a thickness of exactly  $\frac{7}{8}$ ", or has irregular uneven surfaces, bring it to correct size by smoothing against hot "smoothing" iron (10) or hot flat iron, taking care to wipe off any adhering hot rosin remaining from previous application.

#### 11

##### DETERMINATION OF GRADE—OFFICIAL, FIRST ACTION

Compare sample obtained as directed in (a), (b), or (c) with set of duplicates of United States Rosin Standards (11) or with set of type samples or "types" (12), made of rosin, which match United States Rosin Standards, either with or without comparison box (13). To be of given grade the sample of rosin must be equal to or better, that is as light as or lighter in color than the standard for that grade. If grader cannot decide whether sample is equal to, that is, up to the standard or is darker than the standard for a grade, it is given that grade. For example, if sample is being compared with the "N" standard, and grader cannot definitely decide whether it is equal to or is darker than the standard the sample should be graded "N."

### TURPENTINE OIL<sup>3</sup> (SPIRITS OF TURPENTINE)

#### 12

##### COLOR—TENTATIVE

Place in colorimeter a 200 ml flat-bottomed colorimeter tube graduated in mm and filled to depth of 40–50 mm with the turpentine, and on or under it place a No. 2 yellow Lovibond glass. Over or under a second graduated tube in colorimeter, place a No. 1 yellow Lovibond glass and run into it the same turpentine until color matches as nearly as possible color in first tube. Read difference in depth of turpentine in the 2 tubes. If difference is 50–149.9 mm, the turpentine is "standard"; if it is 150 mm or more, the turpentine is "water-white"; and if difference is 25–49.9 mm, the turpentine is "one shade off."

#### 13

##### SPECIFIC GRAVITY—OFFICIAL, FIRST ACTION

Determine sp. gr. at 15.5/15.5° by any convenient method that is accurate within 2 points in fourth place. If determination is made at any other temp., correct reading

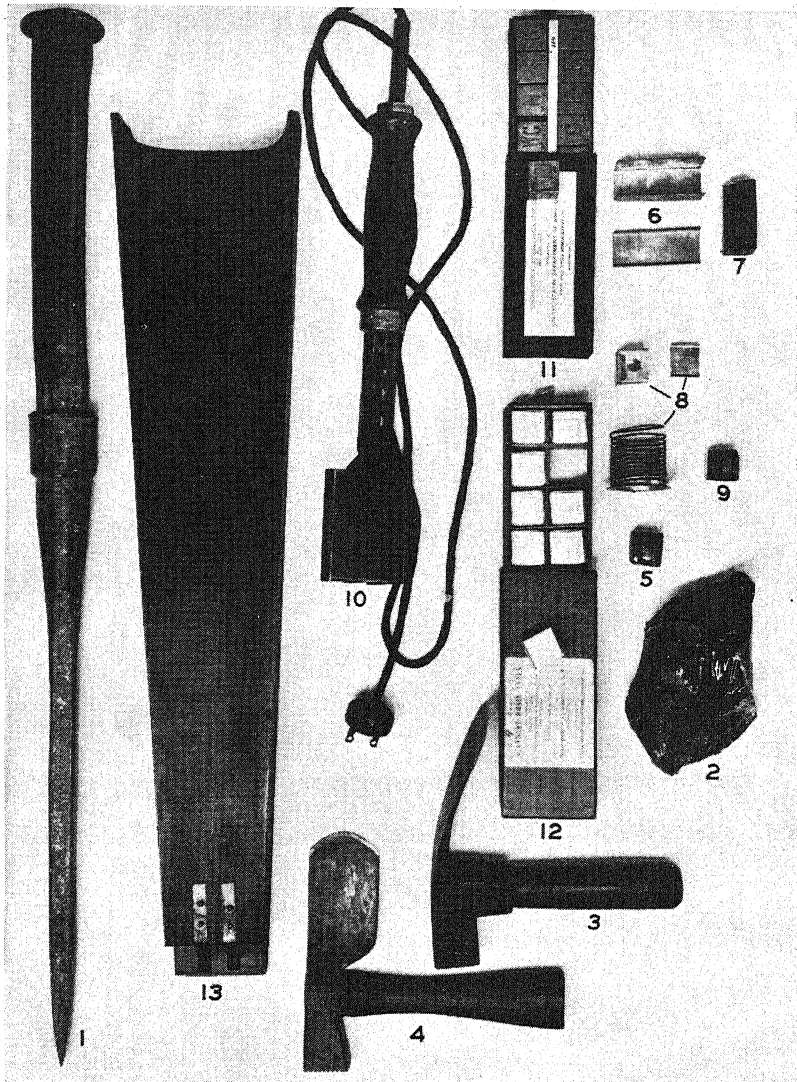


FIG. 12.—ARTICLES USED IN GRADING ROSIN

- |                         |                                        |
|-------------------------|----------------------------------------|
| 1. Spike.               | 8. Short mold.                         |
| 2. Rosin lump.          | 9. Sample taken with it.               |
| 3. Adz.                 | 10. Smoothing iron.                    |
| 4. Hatchet.             | 11. Duplicates, U. S. Rosin Standards. |
| 5. Cut sample rosin.    | 12. Rosin types.                       |
| 6. Long mold.           | 13. Comparison box.                    |
| 7. Sample taken with it |                                        |



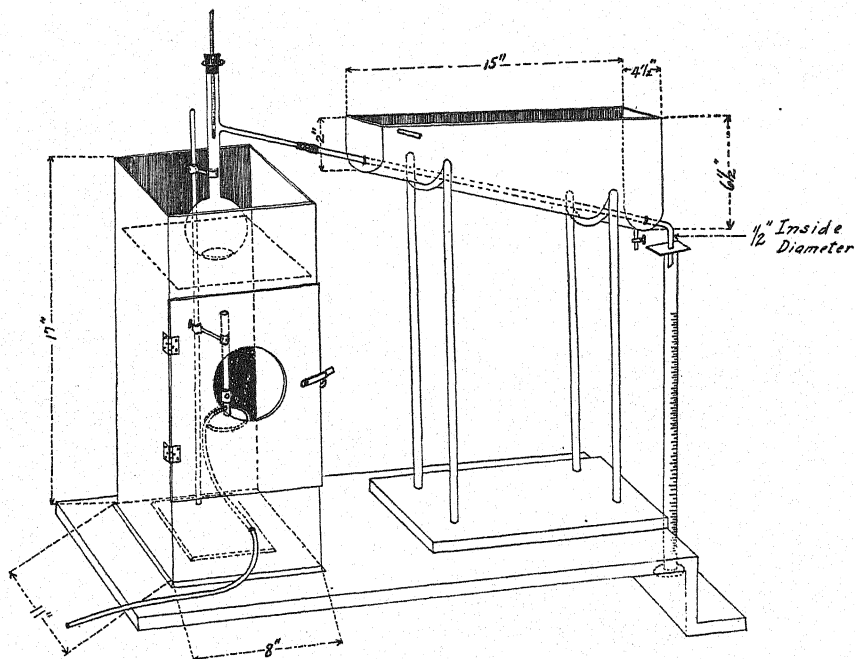


FIG. 13.—APPARATUS FOR DISTILLATION OF TURPENTINE OIL  
OVER OPEN FLAME

by adding thereto or subtracting therefrom 0.00082 for each degree that temp. at which determination is made is respectively above or below  $15.5^{\circ}$ .

#### 14

#### REFRACTIVE INDEX—OFFICIAL, FIRST ACTION

Determine refractive index at any convenient temp., but preferably at  $20^{\circ}$ . If determined at other than  $20^{\circ}$ , calculate result to  $20^{\circ}$  by adding or subtracting the correction factor 0.00045 for each degree that temp. of determination is above or below  $20^{\circ}$ .

#### DISTILLATION—OFFICIAL, FIRST ACTION

#### 15

#### APPARATUS

(a) *Flask*.—Use Engler flask having following dimensions: Diameter of bulb, 6.5 cm; cylindrical neck, 15 cm long, 1.6 cm internal diameter; side or vapor tube, 10 cm long, 0.6 cm external diameter, attached to neck at angle of  $75^{\circ}$ , so that when flask contains its charge of 100 ml of oil the surface of oil shall be 9 cm below bottom of junction of side tube and neck.

Support flask on plate of asbestos 20 cm square, having opening 4 cm in diameter in its center, and heat with open flame; or support flask in metal cup, 15–20 cm in diameter, containing high boiling mineral oil or glycerol and fitted with a concave cover having in center a circular opening  $5\frac{1}{2}$ –6 cm in diameter (Fig. 13). Surround flask and burner with shield to prevent fluctuation in temp. in neck of flask.

(b) *Condenser*.—(1) Use form<sup>4</sup> illustrated in Fig. 13, which consists of thin-walled

brass condenser tubing (No. 20 Stubbs gage seamless)  $\frac{1}{2}$ " inside diameter and 22" long, placed at angle of  $75^\circ$  in metal cooling bath of size and dimensions shown in Fig. 13. Lower end of condenser is cut off at acute angle and curved down for length of 3" so as to project at least  $\frac{1}{2}$ " into receiving cylinder; or, (2) use straight glass condenser 22" long, having 16" in contact with the cooling  $H_2O$  and fitted with adapter, small end of which, cut off at acute angle, is long enough to extend short distance into receiving cylinder, Fig. 14.

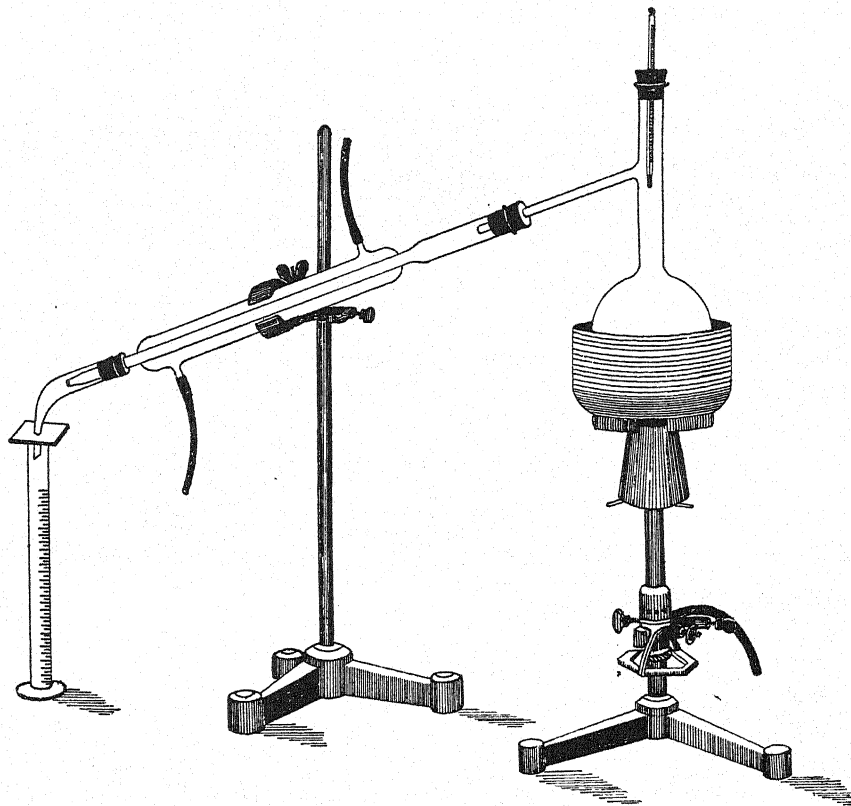


FIG. 14.—APPARATUS FOR DISTILLATION OF TURPENTINE OIL OVER BATH

(c) *Thermometer*.—Use accurate thermometer of Anschütz type, conforming to following specifications: Graduated from  $145$  to  $200^\circ$  in  $0.2^\circ$  intervals. Length, bottom of thermometer to  $175^\circ$  mark, not more than 8 nor less than 6.5 cm; top of bulb to  $145^\circ$  mark, not less than 1.5 cm; from  $145$  to  $175^\circ$  mark, not more than 6 cm. Graduation marks and numbering shall be clear-cut and distinct. Error at any point on scale shall not exceed  $\pm 0.5^\circ$  when tested for total immersion of Hg column.

(d) *Receiving cylinder*.—Use accurately graduated 50–100 ml cylinder. The so-called normal or precision cylinder of 50 ml capacity, having internal diameter of 1.5 cm and graduated in 0.2 ml, is preferred. If cylinder with larger inside diameter is used, place over top pasteboard cover having opening for condenser tube.

Place 100 ml of the turpentine and several small pieces of pumice (or glass) in distilling flask. Fit thermometer so that top of Hg bulb is level with bottom of side tube and 175° mark is below cork. Place flask in position on asbestos board or oil bath and connect with condenser. Apply heat cautiously at first, and when distillation begins so regulate that turpentine distills at rate of not less than 4 nor more than 5 ml per min. (ca 2 drops per second). The initial boiling point is thermometer reading at instant when first drop falls from end of condenser. Discontinue distillation when temp. reaches 170.0°, or equivalent thereof, depending on atmospheric pressure, as determined under 17. Let condenser drain and read percentage distilled. The percentage distilled below successive selected temps. and the temp. at which each successive 10 ml distills may also be determined, if desired, the necessary correction of temp. being made for variations in atmospheric pressure.

17      CORRECTION FOR VARIATION IN ATMOSPHERIC PRESSURE—OFFICIAL,  
FIRST ACTION

The distilling temp. of turpentine is affected 0.057° for each mm variation in barometric pressure. If barometer reading after correcting to 0° is above or below the normal 760 mm, the turpentine will distil at higher or lower temp., respectively, than at normal pressure. Therefore, for each mm that the corrected barometer reading is above 760 mm, correct initial b.p. reading by minus (−)0.057°; and for each mm that corrected barometer reading is below 760 mm, correct initial b.p. reading by plus (+)0.057°. Also correct final temp. observation point (170°) in same way, by adding thereto 0.057° for each mm of pressure above 760 mm, or subtracting therefrom 0.057° for each mm of pressure below 760 mm, as may be required. The actual temp. at which distillation is stopped must be that equivalent to 170° at 760 mm.

MINERAL OIL IN TURPENTINE

*Fuming Sulfuric Acid Method*<sup>5</sup>—Official

*Fuming 38 N sulfuric acid.*—Mix  $\text{H}_2\text{SO}_4$  with sufficient fuming  $\text{H}_2\text{SO}_4$  to obtain a mixture containing slightly more than 82.38% total  $\text{SO}_3$ . If the fuming acid contains 50% excess  $\text{SO}_3$ , ca 100 g of fuming acid to 140 g of concentrated acid will be approximately the correct ratio. Determine exact concentration of mixture and also of a reserve supply of concentrated acid as follows:

Weigh a quantity of the acid in a weighing bulb or pipet having a capillary tube at lower end and a stopcock at upper end and fitted with a Pt wire for suspending on balance. Fill bulb by slight suction and empty lower end of capillary by closing stopcock simultaneously with withdrawal of the capillary from the acid, wiping off first with a moist and then with a dry cloth. Allow acid to flow down sides of neck of a volumetric flask into cold  $\text{H}_2\text{O}$ . (If a flask ca 100 times the volume of the weighing pipet is used, the resultant soln will be near 0.5 N.) Wash all traces of acid into flask, taking precautions to prevent loss of  $\text{SO}_3$  fumes. Make to volume and titrate from a buret against standard alkali, using the indicator with which the alkali was standardized. Calculate  $\text{SO}_3$  content of both acids and add sufficient concentrated acid to the fuming mixture to bring it to 82.38% (100.92% equivalent,  $\text{H}_2\text{SO}_4$ ). The equivalent  $\text{H}_2\text{SO}_4$  content of this acid must not vary more than  $\pm 0.15\%$   $\text{H}_2\text{SO}_4$  from the above figure. Keep acid in small bottles and protect against absorption of moisture from air.

## DETERMINATION

Place 20 ml of the  $\text{H}_2\text{SO}_4$  in a graduated narrow-necked Babcock flask, stopper, and place in ice  $\text{H}_2\text{O}$  to cool. Add slowly from a pipet, a little at a time, 5 ml of the turpentine, gently shaking or rotating flask and keeping temp. at  $60\text{--}65^\circ$  by continued immersion in ice  $\text{H}_2\text{O}$ . When mixture no longer develops heat on shaking, agitate thoroly by vigorously shaking ca  $\frac{1}{2}$  min. Place flask in water bath and heat at  $60\text{--}65^\circ$  for 10 min., keeping contents of flask thoroly mixed by shaking vigorously not less than 6 times during heating period. (CAUTION: If shaking is too vigorous at first, there is danger of escaping  $\text{SO}_2$  forcing some of mixture up over mouth of flask.) Cool to room temp. and fill flask with conc.  $\text{H}_2\text{SO}_4$  until surface rises well into graduated neck. Centrifuge 5 min. at 1200 r.p.m., or 10 min. at 900 r.p.m.; or allow to stand, lightly stoppered, 12 hours. Read volume of unpolymerized residue (middle of meniscus), calculate percentage, record its consistency and color, and determine its refractive index at  $20^\circ$ .

By this method pure gum spirits of turpentine gives less than 2.0% residue, which has a straw or darker color, viscous consistency, and a refractive index of not less than 1.500. A limpid colorless residue with a refractive index of less than 1.500 indicates presence of mineral oil. The unpolymerized residue from an adulterated oil represents 60–80% of total quantity of adulterant present.

## SELECTED REFERENCES

- <sup>1</sup> Ind. Eng. Chem., Anal. Ed., **6**, 122 (1934).
- <sup>2</sup> J. Assoc. Official Agr. Chem., **13**, 48 (1930).
- <sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. **898**; U. S. Bur. Standards Circ. **86**.
- <sup>4</sup> Adopted by American Society for Testing Materials.
- <sup>5</sup> Chem. Ztg., **30**, 631 (1906); U. S. Dept. Agr. Bur. Chem. Circ. **85**; J. Assoc. Official Agr. Chem., **6**, 465 (1923).

## IX. PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

### WHITE LINSEED OIL PAINTS<sup>1</sup>—OFFICIAL

1

#### REAGENTS

(a) *Extraction mixture*.—10 volumes ethyl ether, 6 volumes benzene, 4 volumes methyl alcohol, and 1 volume acetone.

(b) *Aqueous sodium hydroxide soln.*—Dissolve 100 g of NaOH and dilute to 300 ml.

(c) *Alcoholic sodium hydroxide soln.*—Dissolve NaOH in 95% ethyl alcohol in proportion of ca 22 g per 1000 ml. Let stand in stoppered bottle. Decant clear liquid into another bottle and keep well stoppered. This soln should be colorless or only slightly yellow when used; it will keep colorless longer if alcohol is previously treated with NaOH (ca 80 g to 1000 ml), kept at ca 50° 15 days, and then distilled.

(d) *Wij's soln.*—Dissolve I in glacial acetic acid that has m.p. of 14.7–15° and is free from reducing impurities, in such proportion that 13 g of I will be present in 1000 ml of soln. (Preparation of the I monochloride soln presents no great difficulty but it should be done with care and accuracy. There should be in the soln no sensible excess either of I or more particularly of Cl over that required to form the monochloride. This condition is most satisfactorily attained by dissolving in all the acetic acid to be used the requisite quantity of I, using gentle heat to assist soln, if it is found necessary.) Set aside a small portion of this soln, and pass dry Cl into remainder until halogen content of soln is doubled. (Ordinarily, it will be found that by passing the Cl into main part of soln until characteristic color of free I has just been discharged, there will be a slight excess of Cl, which is corrected by the addition of the requisite amount of the unchlorinated portion until all free Cl has been destroyed. A slight excess of I does little or no harm, but excess of Cl must be avoided.)

(e) *Standard sodium thiosulfate soln.*—See XXXI, 18(c).

(f) *Starch soln.*—Stir up 2–3 g of potato starch or 5 g of soluble starch with 100 ml of 1% salicylic acid soln, add 300–400 ml of boiling H<sub>2</sub>O, and boil mixture until starch is practically dissolved, then dilute to 1 liter.

(g) *Potassium iodide soln.*—Dissolve 150 g of KI free from iodate in H<sub>2</sub>O and dilute to 100 ml.

(h) *Acid ammonium acetate soln.*—Mix 150 ml of 80% acetic acid, 100 ml of H<sub>2</sub>O, and 95 ml of NH<sub>4</sub>OH (sp. gr. 0.90).

(i) *Ammonium polysulfide.*—Pass H<sub>2</sub>S gas into 200 ml of NH<sub>4</sub>OH in bottle immersed in running H<sub>2</sub>O or in iced H<sub>2</sub>O until the gas is no longer absorbed; add 200 ml of NH<sub>4</sub>OH and dilute with H<sub>2</sub>O to 1000 ml. Digest this soln with 25 g of flowers of S for several hours and filter.

(j) "*Lead acid.*"—Mix 300 ml of H<sub>2</sub>SO<sub>4</sub> and 1800 ml of H<sub>2</sub>O. Dissolve 1 g of C.P. Pb acetate in 300 ml of H<sub>2</sub>O and add this to the hot soln, stirring meanwhile. Let stand at least 24 hours and siphon thru thick asbestos filter.

(k) *Potassium permanganate soln.*—Dissolve 3.2 g of KMnO<sub>4</sub> in 1 liter of H<sub>2</sub>O, let stand 8–14 days, siphon off clear soln (or filter thru asbestos filter), and standardize as follows: In 400 ml beaker dissolve 0.25–0.30 g (accurately weighed) of Bureau of Standards Na oxalate in 250 ml of hot H<sub>2</sub>O (80–90°) and add 15 ml of H<sub>2</sub>SO<sub>4</sub> (1+1). Titrate at once with the KMnO<sub>4</sub> soln, stirring vigorously and con-

<sup>1</sup> Methods (D 215–29) of the American Society for Testing Materials and adopted tentatively at the 1930 meeting of the A. O. C. These methods have been edited to conform in part to the style of this publication, but otherwise they are as published in the 1929 Supplement to Book of A. S. T. M. Standards. Under the standardization procedure of the A. S. T. M., these methods are under the jurisdiction of the A. S. T. M. Committee D-1 on Preservative Coatings for Structural Materials.

tinuously. Do not add the  $\text{KMnO}_4$  more rapidly than 10–15 ml per min., and add the last 0.5–1 ml dropwise, using particular care to allow each drop to be fully decolorized before the next is introduced. (The temp. of the soln should not be below  $60^\circ$  by time end point is reached. Too rapid cooling may be prevented by allowing beaker to stand on small asbestos-covered hot plate during titration. Use of a small thermometer as stirring rod is most convenient.) Weight of Na oxalate used  $\times 0.833$  = Fe equivalent. Keep the  $\text{KMnO}_4$  soln in glass-stoppered bottle painted black to keep out light.

Fe value of the  $\text{KMnO}_4 \times 1.076$  theoretically equals its Sb equivalent. However, for use in determining Sb, the  $\text{KMnO}_4$  is best standardized as follows: To 0.25 g of pure metallic Sb in 500 ml Pyrex Erlenmeyer flask, add 12–15 ml of  $\text{H}_2\text{SO}_4$  and 10–12 g of  $\text{K}_2\text{SO}_4$ ; heat until all Sb is dissolved, cool, dilute to 250 ml with  $\text{H}_2\text{O}$ , add 20 ml of  $\text{HCl}$ , cool to  $10$ – $15^\circ$ , and titrate with the  $\text{KMnO}_4$  soln until faint pink color is obtained. For special work, after digesting, dilute to 100 ml with  $\text{H}_2\text{O}$ , add 1–2 g of  $\text{Na}_2\text{SO}_3$ , and boil until all  $\text{SO}_2$  is expelled, shown when no blue color is obtained with the starch-iodate paper. Volume will be reduced ca one-half. Dilute to 250 ml with  $\text{H}_2\text{O}$ , add 20 ml of  $\text{HCl}$  (sp. gr. 1.19), and complete titration as described.

(l) *Standard potassium ferrocyanide*.—Dissolve 22 g of the pure salt in  $\text{H}_2\text{O}$  and dilute to 1000 ml. To standardize, transfer ca 0.2 g (accurately weighed) of pure metallic Zn or freshly ignited pure  $\text{ZnO}$  to 400 ml beaker. Dissolve in 10 ml of  $\text{HCl}$  and 20 ml of  $\text{H}_2\text{O}$ . Drop in small piece of litmus paper, add  $\text{NH}_4\text{OH}$  until slightly alkaline, then  $\text{HCl}$  until just acid, and then 3 ml of  $\text{HCl}$ . Dilute to ca 250 ml with hot  $\text{H}_2\text{O}$  and heat nearly to boiling. Run in ferrocyanide soln slowly from buret with constant stirring until a drop tested on white porcelain plate with a drop of the uranyl indicator shows brown tinge after standing 1 min. Run blank with same amounts of reagents and  $\text{H}_2\text{O}$  as used in standardization. Subtract amount of ferrocyanide soln required for blank from amounts used in standardization and in titration of sample. (Standardization must be made under the same conditions of temp., volume, and acidity as obtain when the sample is titrated.)

(m) *Uranyl indicator for zinc titration*.—A 5% soln of uranyl nitrate in  $\text{H}_2\text{O}$  or a 5% soln of uranyl acetate in  $\text{H}_2\text{O}$  made slightly acid with acetic acid.

(n) *Alkaline lead nitrate soln*.—Into 100 ml of  $\text{KOH}$  soln (56 g in 140 ml of  $\text{H}_2\text{O}$ ) pour saturated soln of  $\text{Pb}(\text{NO}_3)_2$  (250 g in 500 ml of  $\text{H}_2\text{O}$ ) until the precipitate ceases to redissolve, stirring constantly while mixing. Let settle, filter thru asbestos, and dilute clear filtrate with an equal volume of  $\text{H}_2\text{O}$ . Use ca 3 vols. of the  $\text{Pb}(\text{NO}_3)_2$  soln for 1 of the  $\text{KOH}$ .

(o) *Ammoniacal cadmium chloride or zinc sulfate soln*.—Dissolve 8 g of  $\text{CdCl}_2$  in 200 ml of  $\text{H}_2\text{O}$  and add 200 ml of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90), or dissolve 50 g of  $\text{ZnSO}_4$  in 270 ml of  $\text{H}_2\text{O}$  and add 230 ml of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90).

(p) *Standard potassium iodate soln*.—Dissolve 3.6 g of  $\text{KIO}_3$  and 39 g of  $\text{KI}$  in 1000 ml of  $\text{H}_2\text{O}$ . (For general work the theoretical S titer of this soln should be used; for special work, soln may be standardized against like material, such as lithopone of known sulfide-S content.) The theoretical titer is based on standard  $\text{Na}_2\text{C}_2\text{O}_4$  and is obtained as follows: To 300 ml of  $\text{H}_2\text{O}$  in 600 ml flask, preferably glass-stoppered, add 10 ml of  $\text{HCl}$  (sp. gr. 1.19) and 1 g of  $\text{KI}$ . Cool, and add 10 ml of 0.1 *N*  $\text{KMnO}_4$  soln that has been standardized against  $\text{Na}_2\text{C}_2\text{O}_4$ . Swirl gently, stopper, and let stand 5 min. Titrate liberated I with standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln until color fades. Add 10 ml of starch soln and continue titration until blue color is destroyed. Repeat titration, substituting 10 ml of iodate soln for the  $\text{KMnO}_4$  soln. Calculate normality of iodate soln.

(q) *Starch indicator for sulfur titration*.—(1) To 1000 ml of boiling  $\text{H}_2\text{O}$ , add a cold suspension of 6 g of starch in 100 ml of  $\text{H}_2\text{O}$  and boil vigorously 5 min. Cool

soln, add 6 g of  $\text{ZnCl}_2$  dissolved in 50 ml of cold  $\text{H}_2\text{O}$ , thoroly mix, and set aside 24 hours. Decant clear supernatant liquid into suitable container, add 3 g of KI, and mix thoroly. (2, optional) Prepare an emulsion of 6 g of soluble starch in 25 ml of  $\text{H}_2\text{O}$ , add a soln of 1 g of NaOH in 10 ml of  $\text{H}_2\text{O}$ , and stir soln until it gelatinizes. Dilute to 1000 ml with  $\text{H}_2\text{O}$ , add 3 g of KI, and mix thoroly.

(r) *Starch-iodate paper*.—Impregnate filter paper with soln obtained by heating 2 g of starch with 100 ml of  $\text{H}_2\text{O}$ , and, after soln, adding 0.2 g of  $\text{KIO}_3$  dissolved in 5 ml of  $\text{H}_2\text{O}$ .

(s) *Standard iodine soln for  $\text{SO}_2$* .—Place 15–20 g of pure KI in liter volumetric flask, dissolve in as little  $\text{H}_2\text{O}$  as possible, and add ca 6.4 g of resublimed I. Shake until all the I is dissolved, dilute to mark with  $\text{H}_2\text{O}$ , and mix. This soln is ca 0.05 N and is standardized against 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$  to obtain its true normality.

(t) *Standard sodium thiosulfate soln for  $\text{SO}_2$* .—Prepare and standardize as directed in (e), using 12.42 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ; or the 0.1 N soln may be diluted with an equal volume of cold  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ .

(u) *Ferric sulfate soln for titanium*.—Prepare soln containing 2% of Fe as ferric sulfate as follows: Dissolve 20 g of pure Fe or plain C steel in slight excess of HCl, oxidize with  $\text{HNO}_3$ , add ca 80 ml of  $\text{H}_2\text{SO}_4$ , and heat until fumes of the latter are evolved. Cool, dilute with  $\text{H}_2\text{O}$  to 1000 ml, digest on steam bath until sulfates are dissolved, and filter if necessary. To oxidize any ferrous Fe that may be present, add 0.1 N  $\text{KMnO}_4$  soln until faint pink color persists 5 min. Ferric ammonium sulfate may also be used.

(v) *Standard ferric sulfate soln for colorimetric determination of iron*.—Determine strength of ferric soln for  $\text{TiO}_2$  determination in terms of Fe and dilute a portion of this soln until one is obtained of the concentration 1 ml = 0.00001 g of Fe.

(w) *Potassium thiocyanate indicator*.—Prepare 2% soln of the pure salt in  $\text{H}_2\text{O}$ .

## 2

### PRELIMINARY PROCEDURE

On receipt of sample make a record of label, noting especially brand, name of manufacturer, and any statement as to composition and net contents. Weigh unbroken package, open, note odor and condition of the contents, pour into clean container, and mix thoroly by pouring from one container to the other, finally leaving the well-mixed sample in the second container, which shall be tightly closed. Use the well-mixed sample at once for determinations described under "Methods." The original can and cover may be cleaned with gasoline, wiped dry, and weighed. This weight subtracted from original weight gives net weight of contents. If desired, the sp. gr. of the paint may be determined and weight per gallon calculated, and volume of paint and capacity of container may be measured.

## 3

### WATER<sup>1</sup>

Mix 100 g of the paint in 250 ml flask with 75 ml of toluene. Place flask in oil bath, connect with condenser, apply heat to bath, and distil until ca 50 ml of distillate has been collected in graduate. Temp. in the flask should then be 105–110°. The number of ml of  $\text{H}_2\text{O}$  collected under the toluene in the receiver is the percentage of  $\text{H}_2\text{O}$  in the paint.

<sup>1</sup> A convenient apparatus for this determination is shown in Fig. 1 (b) of the Standard Method of Test for Water in Petroleum Products and Other Bituminous Materials (A.S.T.M. Designation: D 95–30, 1933 A.S.T.M. Standards, Pt. II, p. 891).

4

## VOLATILE THINNER

Weigh accurately 3–5 g of the paint into tared flat-bottomed dish ca 8 cm in diameter, spreading paint over bottom. Heat at 105–110° 1 hour, cool, and weigh. Calculate loss in weight as percentage of H<sub>2</sub>O and volatile thinner, subtract from this percentage of H<sub>2</sub>O, 3, and report remainder as volatile thinner.

5

## NATURE OF THE THINNER

Transfer ca 150 g of the paint to 500 ml flask fitted with 2-holed cork stopper carrying spray trap connected with vertical condenser. Thru other hole in stopper pass influx tube for steam. (This tube should dip below surface of paint.) Heat flask in oil bath or air bath at 100° and pass thru it current of steam; with steam still passing thru, raise temp. of bath to 130°. Catch distillate in small separatory funnel and continue distillation until 300 ml of H<sub>2</sub>O has been condensed. Portions of this H<sub>2</sub>O may be drawn from cock of separatory funnel from time to time, but care must be taken not to draw out any of volatile thinner. Let distillate stand until it separates into 2 layers, draw off H<sub>2</sub>O, and filter volatile thinner thru dry filter paper into dry flask. If thinner is apparently turpentine, examine distillate as directed in Chap. VIII. If thinner is mixture of turpentine and mineral spirits, an approximate determination of amount of turpentine may be made by the polymerization test specified under turpentine, VIII, 19. It should be noted that turpentine is slightly soluble in H<sub>2</sub>O (ca 0.3–0.4 ml per 100 ml of H<sub>2</sub>O).

To test for benzene, add few drops of distillate to small quantity of mixture of HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>, and heat cautiously. The characteristic odor of nitrobenzene will be noted if benzene is present.

If thinner is apparently all mineral spirits, no further examination is necessary.

If amount of turpentine in thinner is so small that its presence is questioned, it may be detected by placing 2 drops of distillate and 2–3 ml of CHCl<sub>3</sub> in dry test tube and adding 1 drop of SbCl<sub>5</sub>. A slow or slight change in color indicates absence of turpentine. A rapid change in color to dark red or purple indicates possibility of turpentine. The I number for turpentine by Wijs method under these conditions is ca 340. An I number of 20 or over will give additional proof of presence of turpentine and enable calculation of approximate amount.

6

## PERCENTAGE OF PIGMENT

Strain a portion of well-mixed sample thru No. 80 sieve with an opening of .177 mm and wire diameter of .119 mm to remove any skins and weigh accurately ca 15 g of strained paint in weighed centrifuge tube. Add 20–30 ml of extraction mixture, 1(a), mix thoroly with glass rod, wash rod with more of the extraction mixture, and add enough of the reagent to make total of 60 ml in tube. Place tube in container of centrifuge, surround tube with H<sub>2</sub>O, and counterbalance container of opposite arm with a similar tube, or a tube with H<sub>2</sub>O. Whirl at moderate speed until well settled. Decant clear supernatant liquid, repeating extraction twice with 40 ml of extraction mixture and once with 40 ml of ethyl ether. After drawing off ether, set tube in beaker of H<sub>2</sub>O at ca 80° or on top of warm oven 10 min., then in oven at 105–110° 2 hours. Cool, weigh, and calculate percentage of pigment. Grind pigment to fine powder, pass thru No. 80 sieve to remove any skins, and preserve in stoppered bottle.

7

## PERCENTAGE OF NON-VOLATILE VEHICLE

Add together percentages of H<sub>2</sub>O, of volatile thinner, and of pigment, and subtract sum from 100. Report remainder as non-volatile vehicle.



## TESTING NON-VOLATILE VEHICLE

## 8

## PREPARATION OF FATTY ACIDS

(a) To ca 25 g of paint in porcelain casserole, add 15 ml of aqueous NaOH, 1(b), and 75 ml of ethyl alcohol, mix, and heat uncovered on steam bath until all volatile thinner is driven off and saponification is complete. Add 100 ml of  $H_2O$ , boil, add  $H_2SO_4$  (sp. gr. 1.2) (8–10 ml in excess), boil, stir, and transfer to a separatory funnel to which some  $H_2O$  has been added. Draw off as much as possible of acid aqueous layer and any insoluble or precipitated matter, wash once with  $H_2O$  then add 50 ml of  $H_2O$  and 50 ml of ethyl ether. Shake very gently with whirling action to dissolve fatty acids in ether, but not so violently as to form an emulsion. Draw off aqueous layer and wash ether layer with one 15 ml portion of  $H_2O$  and then with 5 ml portions of  $H_2O$  until free from  $H_2SO_4$ . Draw off  $H_2O$  layer completely. Transfer ether soln to dry flask and add 25–50 g of anhydrous  $Na_2SO_4$ . Stopper flask and let stand with occasional shaking at temp. below  $25^\circ$  until  $H_2O$  is completely removed from ether soln, which will be shown by soln becoming perfectly clear above the solid  $Na_2SO_4$ . Decant this clear soln, if necessary, thru dry filter paper into dry 100 ml Erlenmeyer flask. Pass rapid current of dry air (thru a  $CaCl_2$  tower) into mouth of the Erlenmeyer flask and heat to temp. below  $75^\circ$  on dry hot plate until ether is entirely driven off. (It is important to follow all details, as ether generally contains alcohol, and after washing with  $H_2O$  always contains  $H_2O$ . It is difficult to remove  $H_2O$  and alcohol by evaporation from fatty acids, but washing of the ether soln and subsequent drying with anhydrous  $Na_2SO_4$  remove both  $H_2O$  and alcohol. Ether, in the absence of  $H_2O$  and alcohol, is easily removed from fatty acids by gentle heat. If pigment settles out rapidly in a sample of the paint on standing so that sufficient vehicle can be poured off, or if sufficient vehicle is obtained by centrifuging the paint, it will be advantageous to saponify this separated vehicle and liberate and prepare the fatty acids as described.)

(b) Instead of procedure (a) the following may be used, especially with samples that give trouble by (a): To ca 50 g of paint in porcelain casserole, add 30 ml of aqueous 30% NaOH and 125 ml of ethyl alcohol, mix, and evaporate on steam bath until residue is dry. Transfer to 400 ml beaker and boil with 200 ml of  $H_2O$ , add  $H_2SO_4$  (sp. gr. 1.2) (25 ml in excess), boil, stir, filter thru large coarse paper, and drain. Scrape mass into flask, shake violently with ether, centrifuge, decant into separatory funnel, and wash with small amounts of  $H_2O$  until free of  $H_2SO_4$ . Transfer ether soln to dry flask containing ca 40 g of anhydrous  $Na_2SO_4$  and allow to stand until ether layer is clear. Decant clear soln thru filter paper into dry 100 ml flask. Pass rapid current of dry air into mouth of flask and heat to temp. below  $75^\circ$  on dry hot plate until ether is entirely removed. Keep these prepared fatty acids in stoppered flask and examine at once.

These methods of preparing the fatty acids directly from the material, rather than from the extracted vehicle, are based upon past experience in sometimes obtaining too low results by the latter method. Occasionally, however, trouble is experienced in saponifying the entire material, due to interference of pigment. In this case it is permissible to save the extracted vehicle (6), evaporate the organic solvents on steam bath, and saponify and prepare the fatty acids in the usual manner from this extract. If the I number obtained in this manner passes a given specification, no further work is necessary; if the I number is low, it will be necessary to repeat work directly on entire material.

The fatty acids prepared as above should be placed in stoppered flask and examined at once.

9

## TEST FOR MINERAL OIL AND OTHER UNSAPONIFIABLE MATTER

Place 10 drops of the fatty acids, 8, in a 50 ml test tube, add 5 ml of alcoholic soda, 1(c), boil vigorously 5 min., add 40 ml of  $H_2O$ , and mix. A clear soln indicates that not more than traces of unsaponifiable matter are present.

10

IODINE NUMBER OF FATTY ACIDS<sup>1</sup>

Place small quantity of the fatty acids, 8, in small weighing buret or beaker. Weigh accurately. Transfer by dropping ca 0.15 g (0.10–0.20 g) into 500 ml bottle having well-ground glass stopper, or Erlenmeyer flask having a specially flanged neck for the I test. Reweigh buret or beaker and determine amount of sample used. (If desired, sample may be weighed in small wide-mouthed vial and the vial containing weighed sample placed in bottle or flask.) Add 10 ml of  $CHCl_3$ . Whirl bottle or flask to dissolve sample. Add 10 ml of  $CHCl_3$  to 2 empty bottles or flasks like flask used for sample. Add to each bottle or flask 25 ml of the Wijs soln, 1(d), and let stand with occasional shaking 1 hour in dark place at temp. 21–23°. Add 10 ml of KI soln, 1(g), and 100 ml of  $H_2O$ , and titrate with standard  $Na_2S_2O_3$  soln, 1(e), using starch as indicator. The titrations on the 2 blank tests should agree within 0.1 ml. From difference between average of blank titrations and titration on sample and I value of thiosulfate soln, calculate I number of sample tested, and report as I number in centigrams of I to 1 g of sample.

## ROSIN

11

*Liebermann-Storch Test<sup>2</sup>*

To ca 1 g of fatty acids add 15 ml of acetic anhydride and shake until soln is complete. Pour few drops of this soln on white porcelain plate (a crucible cover serves well) and add drop of  $H_2SO_4$  (sp. gr. 1.53). A fugitive violet color indicates rosin.

12

*Halphen-Hicks Test<sup>3</sup>*

Test the fatty acids with Halphen-Hicks reagent as follows:

*Soln A.*—Dissolve 1 part by volume of phenol in 2 parts by volume of  $CCl_4$ .

*Soln B.*—Dissolve 1 part by volume of Br in 4 parts by volume of  $CCl_4$ .

Add 1–2 ml of Soln A to ca 1 g of fatty acids and pour this mixture into cavity of an ordinary porcelain color-reaction plate until it just fills depression. Immediately fill adjacent cavity with Soln B. Cover plate with inverted watch-glass and note color, if any, produced in former soln by action of Br vapors from Soln B. A decided purple or deep indigo blue color is indication of presence of rosin.

## PIGMENT

13

*Qualitative and Quantitative Examination*

A complete qualitative analysis, following well-established method, should be made and the quantitative scheme modified as required. Add acetic acid slowly to pigment until all carbonate is decomposed (noting whether any  $H_2S$  is evolved); add large excess of acid  $NH_4$  acetate soln, 1(h), boil, filter, and test filtrate for metals other than Pb and Zn (especially Ca and Ba). Absence of Ca in this filtrate indicates

<sup>1</sup> If appreciable amounts of rosin or of unsaponifiable matter are found to be absent in the vehicle of a paint, the I number of the fatty acids gives best indication (tho not proof) of presence of linseed oil. An I number of less than 175 (Wijs) for fatty acids is indication that the non-volatile vehicle was not pure linseed oil.

<sup>2</sup> Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes*, Vol. 1, p. 623 (1921).

<sup>3</sup> *J. Ind. Eng. Chem.*, 3, 86 (1911).

that extending pigments contain no  $\text{CaCO}_3$  or  $\text{CaSO}_4$ ; absence of Ba indicates that extending pigments contain no  $\text{BaCO}_3$ .<sup>1</sup> Wash matter insoluble in acid  $\text{NH}_4$  acetate soln with another portion of this soln, and finally with hot  $\text{H}_2\text{O}$ . Dry, ignite, and test this insoluble matter for siliceous matter,  $\text{BaSO}_4$ , and Ti compounds. To test for Ti compounds, place small amount of the insoluble matter, or of original sample (ca 0.5 g), in 250 ml Pyrex glass beaker, and add 20 ml of  $\text{H}_2\text{SO}_4$  and 7–8 g of  $(\text{NH}_4)_2\text{SO}_4$ . Mix well, and boil few minutes. A residue denotes presence of  $\text{SiO}_2$  or siliceous matter. Cool soln, dilute with 100 ml of  $\text{H}_2\text{O}$ , heat to boiling, settle, filter, and wash with hot 5%  $\text{H}_2\text{SO}_4$  until free from Ti. (The residue may be tested for Pb, Ba, and  $\text{SiO}_2$ .) Add  $\text{H}_2\text{O}_2$  to small portion of filtrate; a clear yellow-orange color indicates presence of Ti. Boil another portion of filtrate with metallic Sn or Zn (a pale blue to violet coloration indicates Ti). Treat another portion (ca 1 g) of pigment with 20 ml of  $\text{HCl}$  (1+1) and note whether any  $\text{H}_2\text{S}$  is evolved; boil soln ca 5 min., add ca 25 ml of hot  $\text{H}_2\text{O}$ , filter, and wash with hot  $\text{H}_2\text{O}$ . Render small portion of filtrate alkaline with  $\text{NH}_4\text{OH}$ , acidify with  $\text{HCl}$ , and add a little  $\text{BaCl}_2$  soln; a white precipitate ( $\text{BaSO}_4$ ) indicates presence of a soluble sulfate. To another portion of filtrate add a little  $\text{H}_2\text{SO}_4$  (a white precipitate indicates presence of Pb, soluble Ba, or both (some  $\text{CaSO}_4$  may also separate)); filter, wash to remove free acid, and treat precipitate with a few drops of  $\text{KI}$  soln (formation of yellow  $\text{PbI}_2$  indicates presence of Pb). The white precipitate may also be treated with  $\text{H}_2\text{S}$  water (formation of black  $\text{PbS}$  indicates presence of Pb). To another portion of original filtrate add  $\text{NH}_4\text{OH}$  until alkaline, render slightly acid with acetic acid, heat to boiling, and add a little  $\text{K}_2\text{Cr}_2\text{O}_7$  soln (a yellow or orange-yellow precipitate indicates presence of Pb, soluble Ba, or both). To another portion of original filtrate add a few drops of  $\text{K}_4\text{Fe}(\text{CN})_6$  soln (a white precipitate with bluish tinge indicates presence of Zn). Pass into remaining portion of original filtrate current of  $\text{H}_2\text{S}$  5–10 min., add an equal volume of  $\text{H}_2\text{O}$ , and pass  $\text{H}_2\text{S}$  into soln ca 5 min.; filter, wash with  $\text{H}_2\text{S}$  water; then digest precipitate with  $\text{NH}_4$  polysulfide, filter, acidify with  $\text{HCl}$ , and warm (presence of Sb is indicated by the separation of an orange colored precipitate). The filtrate from the  $\text{H}_2\text{S}$  precipitate may be tested for Ba, Ca, and Mg in usual manner.

## 14

## SPECIFIC GRAVITY

If sp. gr. of pigment is required, determine according to the standard Methods of Test for Specific Gravity of Pigments (Serial Designation: D 153–27) of American Society for Testing Materials.<sup>2</sup>

## SINGLE PIGMENTS

## 15

## BASIC CARBONATE OF LEAD

(a) *Total Lead (Gravimetric)*.—Dissolve 1 g in 20 ml of  $\text{HNO}_3$  (1+1) in covered beaker, heating till all  $\text{CO}_2$  is expelled. Wash off cover, add 20 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate to fumes of  $\text{SO}_3$ , cool, and add ca 150 ml of  $\text{H}_2\text{O}$  and 150 ml of alcohol. Let stand in cold  $\text{H}_2\text{O}$  1 hour, filter on Gooch crucible, wash with alcohol, dry at  $110^\circ$ , and weigh  $\text{PbSO}_4$ . Calculate to  $\text{PbO}$  or to basic carbonate.<sup>3</sup> Instead of determining Pb as sulfate, sample may be dissolved by boiling with acetic acid; then dilute to ca 200 ml with  $\text{H}_2\text{O}$ , make alkaline with  $\text{NH}_4\text{OH}$ , then acid with acetic

<sup>1</sup> If original sample contained  $\text{BaCO}_3$  and  $\text{PbSO}_4$ ,  $\text{CaSO}_4$  or other soluble sulfate, the soluble Ba will form with the soluble sulfate a precipitate of  $\text{BaSO}_4$ , which will be determined as "insoluble matter." If sample contained  $\text{SrSO}_4$  or  $\text{SrCO}_3$ , some  $\text{SrSO}_4$  may be counted as  $\text{BaSO}_4$ , some Sr will count as soluble Ba, and some may be counted as CaO. This element is not separated, as it probably will not be encountered, or will be present as an impurity in the Ba and Ca compounds.

<sup>2</sup> 1933 A.S.T.M. Standards, Part II, p. 568.

<sup>3</sup> This method of weighing  $\text{PbSO}_4$  is not accurate in presence of Ca compounds.

acid, heat to boiling, and add 10–15 ml of 10% soln of  $K_2Cr_2O_7$ . Heat till yellow precipitate assumes orange color. Let settle and filter on Gooch crucible, washing by decantation with hot  $H_2O$  till washings are colorless, finally transferring all precipitate. Wash with alcohol and then with ether; dry at  $110^\circ$  and weigh  $PbCrO_4$ . (Any insoluble matter should be filtered out before precipitating the Pb.)

(b) *Total Lead (Volumetric)*.—Dissolve 0.5 g of sample in 10 ml of  $HCl$ , boil till soln is effected, cool, dilute to 40 ml, and neutralize with  $NH_4OH$ . Add acetic acid until distinctly acid, dilute to 200 ml with hot  $H_2O$ , boil, and titrate with  $NH_4$  molybdate as follows:

Dissolve 4.25 g of  $NH_4$  molybdate in  $H_2O$  and make to 1 liter. To standardize this soln, dissolve ca 0.2 g of pure Pb foil in  $HNO_3$  (pure  $PbO$  or  $PbSO_4$  may also be used), evaporate nearly to dryness, add 30 ml of  $H_2O$ , then 5 ml of  $H_2SO_4$  (sp. gr. 1.84), cool, and filter. Drop filter with  $PbSO_4$  into flask, add 10 ml of  $HCl$ , boil till completely disintegrated, and add 15 ml of  $HCl$ , 25 ml of  $H_2O$ , and  $NH_4OH$  till alkaline. Acidify with acetic acid, dilute to 200 ml with hot  $H_2O$ , and boil. Titrate, using an outside indicator of 1 part of tannic acid in 300 parts of  $H_2O$ .

It should be noted that when Ca is present, it forms a more or less insoluble molybdate, and results may be high. With samples containing less than 10% of Pb, the Pb should be precipitated as  $PbSO_4$ , filtered, redissolved, and titrated as in process of standardizing.

(c) *Lead Carbonate and Lead Hydroxide*.—Determine  $CO_2$  by evolution with  $HCl$  (1+1), absorbing in soda lime or  $KOH$  soln. Calculate  $CO_2$  to  $PbCO_3$ , subtract  $PbO$  equivalent from total  $PbO$ , and calculate residual  $PbO$  to  $Pb(OH)_2$ .

The following method of A. N. Finn (unpublished) gives total basicity of a pure white lead: Place 2 g of pigment in an evolution flask, add a little  $CO_2$ -free  $H_2O$  and connect to separatory funnel and condenser (Knorr type). Add thru funnel, finally washing down, 100 ml of 0.25  $N$   $HNO_3$ , boil, and absorb  $CO_2$  in soda-lime tube in usual manner (having  $H_2SO_4$  and  $CaCl_2$  drying tubes in train), and weigh. To soln in evolution flask add ca 20 ml of neutral  $Na_2SO_4$  soln and titrate with 0.25  $N$   $NaOH$  soln (carbonate-free), using phenolphthalein. Calculate  $CO_2$  to  $PbCO_3$ . Calculate amount of 0.25  $N$  acid corresponding to the  $CO_2$  and deduct from total amount of 0.25  $N$  acid neutralized by sample. Calculate difference to  $Pb(OH)_2$ .

## 16

### BASIC SULFATE OF LEAD

(a) *Qualitative Analysis*.—Test for matter insoluble in acid  $NH_4$  acetate soln, 1(h), for Ca, for carbonates, and for any other impurities suspected, by regular methods of qualitative analysis.

(b) *Moisture*.—Place 1 g of sample in tared, wide-mouthed, short weighing tube provided with glass stopper. Heat with stopper removed 2 hours at  $105$ – $110^\circ$ . Insert stopper, cool, and weigh. Calculate loss in weight as moisture.

(c) *Insoluble Impurity and Total Lead*.—In 250 ml beaker, moisten 1 g of pigment with a few drops of alcohol, and add 50 ml of the acid  $NH_4$  acetate soln. Heat to boiling and boil 2 min. Decant thru filter paper, leaving any undecomposed matter in beaker. To residue in beaker, add 50 ml of the acid  $NH_4$  acetate soln, heat to boiling, and boil 2 min. Filter thru same paper and wash with hot  $H_2O$ . If an appreciable residue remains, ignite and weigh as insoluble impurity. Unite the acid  $NH_4$  acetate solns, heat to boiling, and add dropwise, while stirring, a slight excess (10–15 ml) of 10% soln of dichromate. Heat until precipitate assumes orange color, let settle, filter on weighed Gooch crucible, wash by decantation with hot  $H_2O$  until washings are colorless, and finally transfer all precipitate to crucible. Wash with 10 ml of 95% ethyl alcohol and finally with 10 ml of ethyl ether. Dry at  $105$ – $110^\circ$ , cool, and weigh  $PbCrO_4$ . Calculate to  $PbO$  by multiplying by factor 0.69.

(d) *Zinc Oxide*.—Weigh accurately ca 1 g of pigment, transfer to 400 ml beaker, add 30 ml of HCl (1+2), and boil 2-3 min. Add 200 ml of H<sub>2</sub>O and small piece of litmus paper. Add NH<sub>4</sub>OH until slightly alkaline, render just acid with HCl, add 3 ml of HCl, heat nearly to boiling, and titrate with standard potassium ferrocyanide as in standardizing that soln, 1(l). Calculate total Zn as ZnO.

(e) *Lead Sulfate*.—Treat 0.5 g of pigment in 400 ml beaker with a few drops of alcohol, add 10 ml of Br water, 10 ml of HCl (1+1), and 3 g of NH<sub>4</sub>Cl. Cover with watch-glass and heat on steam bath 5 min. Add hot H<sub>2</sub>O to give total volume of ca 200 ml, boil 5 min., filter to separate any insoluble matter (a pure pigment should be completely dissolved), and wash thoroly with hot H<sub>2</sub>O. (Insoluble matter may be ignited, weighed, and examined qualitatively.) Neutralize clear soln (original soln or filtrate from insoluble matter) in covered beaker with dry Na<sub>2</sub>CO<sub>3</sub>, add 1 g more of dry Na<sub>2</sub>CO<sub>3</sub>, and boil 10-15 min. Wash off cover, let settle, filter, and wash with hot H<sub>2</sub>O. Redissolve precipitate in HCl (1+1), reprecipitate with Na<sub>2</sub>CO<sub>3</sub> as above, filter, and wash thoroly with hot H<sub>2</sub>O. Acidify united filtrates with HCl, adding ca 1 ml in excess. Boil to expel Br, and to clear boiling soln add slowly, while stirring, 15 ml of 10% BaCl<sub>2</sub> soln. Let stand on steam bath ca 1 hour, filter on weighed Gooch crucible, wash thoroly with boiling H<sub>2</sub>O, dry, ignite, cool, and weigh as BaSO<sub>4</sub>. Calculate to PbSO<sub>4</sub>, using factor 1.3.

(f) *Calculations*.—Calculate percentage of PbSO<sub>4</sub> to PbO by multiplying by factor 0.736 and subtract result from percentage of PbO found under (c), reporting difference as PbO. Report ZnO found under (d) as percentage of ZnO. Report moisture and insoluble matter as such.

## 17

## ZINC OXIDE

(a) *Total Zinc*.—Dissolve 0.25-0.3 g in 10 ml of HCl and 20 ml of H<sub>2</sub>O; make alkaline with NH<sub>4</sub>OH, then acid with HCl; add 3 ml more of HCl, dilute to ca 250 ml with H<sub>2</sub>O, heat nearly to boiling, and titrate with standard K<sub>4</sub>Fe(CN)<sub>6</sub> soln as in standardizing that soln, 1(l). Mn, Fe, and Cu interfere. If they are present in sample, remove as follows: Add to cool HCl soln of Zn 35 ml of a prepared soln of NH<sub>4</sub>OH and NH<sub>4</sub>Cl (50 ml NH<sub>4</sub>OH, 20 g NH<sub>4</sub>Cl, and 75 ml H<sub>2</sub>O). Boil soln very gently 1-2 min. Add saturated Br water and continue boiling a short time. Filter hot soln and wash precipitate 10 times with nearly boiling NH<sub>4</sub>Cl mixture (100 g NH<sub>4</sub>Cl and 50 ml NH<sub>4</sub>OH made up to 1 liter with H<sub>2</sub>O). Drop small piece of litmus paper in filtrate and cautiously neutralize with HCl, finally adding 3 ml in excess. Dilute, if necessary, to ca 200 ml with hot H<sub>2</sub>O, heat nearly to boiling, and add 50 ml of saturated H<sub>2</sub>S water. Titrate with the K<sub>4</sub>Fe(CN)<sub>6</sub> soln.

(b) *Total Soluble Sulfur*.<sup>1</sup>—Moisten 10 g sample with H<sub>2</sub>O, add few drops of Br and then HCl, boil to expel Br, filter from any insoluble matter, and wash with hot H<sub>2</sub>O. Make alkaline with NH<sub>4</sub>OH, then just slightly acid with HCl, heat to boiling, and add ca 15 ml of hot BaCl<sub>2</sub> soln. Let stand several hours (overnight), filter on weighed Gooch crucible, wash well with hot H<sub>2</sub>O, dry, ignite 5 min., cool, and weigh as BaSO<sub>4</sub>. Calculate to S.

## 18

## LITHOPONE

(Ponolith, Jersey Lily White, Becton White, Charlton White, Orr's White)

(a) *Insoluble and Total Zinc*.—Take 1 g of sample in 200 ml beaker, add 10 ml of HCl, mix, and add in small portions ca 1 g of KClO<sub>3</sub>. Heat on steam bath until ca half of liquid is evaporated. Dilute with H<sub>2</sub>O, add 5 ml of H<sub>2</sub>SO<sub>4</sub> (1+10), boil,

<sup>1</sup> Method of G. Rigg.

let settle, filter, wash, ignite, cool, and weigh insoluble (should be only  $\text{BaSO}_4$ ). Make qualitative examination for  $\text{Al}_2\text{O}_3$  and  $\text{SiO}_2$ . Examine insoluble under microscope for presence of natural crystalline barytes. (Sample may also be examined direct.) Make filtrate from insoluble alkaline with  $\text{NH}_4\text{OH}$ , then acid with  $\text{HCl}$ ; add 3 ml more of  $\text{HCl}$ , dilute to ca 250 ml with  $\text{H}_2\text{O}$ , heat nearly to boiling, and titrate with  $\text{K}_4\text{Fe}(\text{CN})_6$  soln as directed under 17. Calculate to Zn.

(b) *Zinc Oxide*.—Weigh accurately 1 g of the lithopone, transfer to 250 ml beaker (moisten with a few drops of alcohol if an extracted pigment), add ca 100 ml of 1–3% acetic acid, stir vigorously but *do not heat*, cover, and let stand 18 hours, stirring once every 5 min. for first 30 min. Filter, wash with 1–3% acetic acid followed by  $\text{H}_2\text{O}$  until washings give no test for Zn with the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. Dilute clear filtrate to ca 200 ml with  $\text{H}_2\text{O}$  and add 30 ml of  $\text{HCl}$  (1+2) and small piece of litmus paper; add  $\text{NH}_4\text{OH}$  until slightly alkaline and render just acid with  $\text{HCl}$ ; add 3 ml of  $\text{HCl}$ , heat nearly to boiling, and titrate with the standard  $\text{K}_4\text{Fe}(\text{CN})_6$  soln as directed under 1(l). Calculate to ZnO. Calculate result to Zn, subtract from total Zn, and calculate difference to ZnS. (Any  $\text{ZnCO}_3$  or  $\text{ZnSO}_4$  is included in the ZnO.)

(c) *Zinc Sulfide*.<sup>1</sup>—Place 0.5 g of pigment in evolution flask with ca 10 g of “feathered” or mossy Zn, add 50 ml of  $\text{H}_2\text{O}$ , and insert stopper carrying a separator and an exit tube. Run in 50 ml of  $\text{HCl}$  from funnel, having previously connected exit tube to 2 absorption flasks in series; first flask contains 100 ml of alkaline  $\text{Pb}(\text{NO}_3)_2$  soln; second flask, 50 ml of same as safety device. After all the acid has run into evolution flask, heat slowly, finally boiling until first appearance of steam in first absorption flask; disconnect, let  $\text{PbS}$  settle, filter, and wash with cold  $\text{H}_2\text{O}$ , then with hot  $\text{H}_2\text{O}$  till neutral to litmus paper and washings give no test for Pb. Dissolve the  $\text{PbS}$  precipitate in hot, dilute  $\text{HNO}_3$ , evaporate to fumes with  $\text{H}_2\text{SO}_4$ , and finally weigh as  $\text{PbSO}_4$ . Calculate  $\text{PbS}$  or  $\text{PbSO}_4$  to ZnS.

Make the alkaline Pb soln as follows: Into 100 ml of  $\text{KOH}$  soln (56 g in 140 ml of  $\text{H}_2\text{O}$ ) pour saturated soln of  $\text{Pb}(\text{NO}_3)_2$  (250 g in 500 ml of  $\text{H}_2\text{O}$ ) until precipitate ceases to redissolve, stirring constantly while mixing (ca 3 volumes of Pb soln will be required for 1 of alkali).

Instead of absorbing the evolved  $\text{H}_2\text{S}$  in alkaline  $\text{Pb}(\text{NO}_3)_2$  soln a soln of 8 g of  $\text{CdCl}_2$  in 250 ml of  $\text{H}_2\text{O}$  and 150 ml of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90) may be used. Filter the  $\text{CdS}$  precipitate on weighed Gooch, wash with  $\text{H}_2\text{O}$  containing a little  $\text{NH}_4\text{OH}$ , dry at  $100^\circ$ , and weigh. Calculate to ZnS. It is better to filter the  $\text{CdS}$  on small filter and wash as above, then place filter and precipitate in beaker and dissolve in  $\text{HCl}$  and  $\text{KClO}_3$  (keeping at room temp. at first); filter out any paper pulp or insoluble matter; make filtrate alkaline with  $\text{NH}_4\text{OH}$ , then just acid with  $\text{HCl}$ , heat to boiling, and precipitate with  $\text{BaCl}_2$  in usual manner. Filter, wash, ignite, and weigh  $\text{BaSO}_4$ . Calculate to ZnS.

For very rapid work wash contents of absorption flask, after all  $\text{H}_2\text{S}$  has been absorbed, into vessel with *cold*  $\text{H}_2\text{O}$  and diluted to ca 1 liter, acidify with  $\text{HCl}$ , and titrate with standard I soln. Use starch indicator. (The precipitate should be completely dissolved.) Prepare I soln by dissolving ca 12.7 g of pure resublimed I and 18 g of KI in a little  $\text{H}_2\text{O}$  and diluting to 1 liter.

## 19

## TITANIUM PIGMENT

(a) *Titanium Oxide*.—Transfer 0.5 g of dried sample to 250 ml Pyrex beaker and add 20 ml of  $\text{H}_2\text{SO}_4$  and 7–8 g of  $(\text{NH}_4)_2\text{SO}_4$ . Mix well and heat on hot plate until fumes of  $\text{H}_2\text{SO}_4$  are evolved; continue heating over strong flame until soln is com-

<sup>1</sup> Evolution method of W. G. Scott, *White Paints and Painting Material*, p. 257; see also Blair, *Chemical Analysis of Iron*.

plete (ca 5 min. of boiling) or it is apparent that residue is composed of  $\text{SiO}_2$  or siliceous matter. Use caution in visually examining this hot soln. Cool soln, dilute with 100 ml of  $\text{H}_2\text{O}$ , stir, heat carefully to boiling while stirring, let settle, filter thru paper, and transfer entire precipitate to paper. Wash insoluble residue with cold 5% (by volume)  $\text{H}_2\text{SO}_4$  until Ti is removed.

Dilute filtrate to 200 ml and add ca 10 ml of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90) to lower acidity to ca 5%  $\text{H}_2\text{SO}_4$  (by volume).

Wash out Jones reductor<sup>1</sup> with dilute 5% by volume  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}$ , leaving sufficient  $\text{H}_2\text{O}$  in reductor to fill to upper level of the Zn. (These washings should require not more than 1 or 2 drops of 0.1 N  $\text{KMnO}_4$  soln to obtain pink color.) Empty receiver, and put in it 25 ml (measured in graduate) of ferric sulfate soln, 1(u). Reduce prepared Ti soln as follows:<sup>2</sup>

(1) Run 50 ml of the 5%  $\text{H}_2\text{SO}_4$  soln thru reductor at ca 100 ml per min.

(2) Follow with Ti soln.

(3) Wash out with 100 ml of 5%  $\text{H}_2\text{SO}_4$ .

(4) Finally run thru ca 100 ml of  $\text{H}_2\text{O}$ . See that reductor is always filled with soln or  $\text{H}_2\text{O}$  to upper level of the Zn. Gradually release suction, thoroly wash glass tube that was immersed in the ferric sulfate soln, remove receiver, and titrate immediately with 0.1 N  $\text{KMnO}_4$  soln. 1 ml of 0.1 N  $\text{KMnO}_4 = 0.0048$  g of Ti or 0.008 g of  $\text{TiO}_2$ . Run blank determination, using same reagents and washing reductor as in above determination. Subtract this permanganate reading from original reading and calculate final reading to  $\text{TiO}_2$  (will include Fe, Cr, As, and any other substance reduced by Zn and acid). See calculations under (b) for reporting  $\text{TiO}_2$ .

(b) *Iron Oxide*.—Weigh 1 g of sample and treat as directed in (a) as far as “dilute with 100 ml of  $\text{H}_2\text{O}$ ,” 5th line; transfer without filtering to graduated 200 ml flask, cool, fill to mark with  $\text{H}_2\text{O}$ , mix, let settle, and determine Fe colorimetrically as follows: Filter thru dry filter paper, discarding first 20 ml. Transfer 50 ml of clear filtrate to clean 100 ml Nessler tube or other comparator. Add a drop or two of 0.1 N  $\text{KMnO}_4$  soln to oxidize any ferrous Fe. The faint pink color should persist at least 5 min. Add 10 ml of  $\text{KCNS}$  or  $(\text{NH}_4)\text{CNS}$  soln, 1(w), dilute with  $\text{H}_2\text{O}$  to 100 ml, and mix thoroly. Compare color immediately with a series of standards, prepared side by side with sample, in similar tubes. Prepare standards from the standard ferric sulfate soln, 1(v), so as to have a range of from 0.000005 to 0.00004 g Fe (0.5–4.0 ml). Transfer desired volumes of the standard ferric sulfate soln to 100 ml Nessler tubes containing 50 ml each of acid soln made up by dissolving 8 g of  $(\text{NH}_4)_2\text{SO}_4$  in  $\text{H}_2\text{O}$ , adding 20 ml of  $\text{H}_2\text{SO}_4$ , cooling, diluting with  $\text{H}_2\text{O}$  to 200 ml, and mixing. Add a drop of 0.1 N  $\text{KMnO}_4$  soln (or sufficient to yield pink color that will persist 5 min.), and then 10 ml of the thiocyanate soln. Finally dilute all standards with  $\text{H}_2\text{O}$  to 100 ml and mix each thoroly.

NOTE.—For a single sample it is more convenient to run the standard Fe soln from buret into Nessler tube, containing 50 ml of acid soln (made by dissolving 8 g of  $(\text{NH}_4)_2\text{SO}_4$  in  $\text{H}_2\text{O}$ , adding 20 ml of  $\text{H}_2\text{SO}_4$ , cooling, diluting with  $\text{H}_2\text{O}$  to 200 ml, and mixing), a drop of 0.1 N  $\text{KMnO}_2$  soln, 10 ml of the thiocyanate soln, and then dilute with  $\text{H}_2\text{O}$  until depth of color produced after diluting to 100 ml and mixing, exactly matches that of sample. From buret reading calculate amount of Fe. When using standards, make the color comparisons immediately.

Report total Fe found as  $\text{Fe}_2\text{O}_3$ . Calculate  $\text{TiO}_2$  equivalent by multiplying  $\text{Fe}_2\text{O}_3$  result by factor 1.003 and subtract this figure from total  $\text{TiO}_2$  as determined in (a) and report remainder as  $\text{TiO}_2$ .

Report all results on dry or moisture-free basis.

<sup>1</sup> Directions for preparing a Jones reductor may be found in Blair, *The Chemical Analysis of Iron*, 8th Ed., pp. 88–89, or Treadwell-Hall, *Analytical Chemistry*, Vol. 2, 5th Ed.

<sup>2</sup> Lundell and Knowles, *J. Am. Chem. Soc.*, 45, 2620 (1923).

## MIXED OR COMPOSITE PIGMENTS

20

MOISTURE<sup>1</sup> (MATTER VOLATILE AT 105–110°)

Place 1–2 g of sample in a wide-mouthed, short weighing tube provided with glass stopper. Heat with stopper removed 2 hours at 105–110°. Insert stopper, cool, and weigh. Calculate loss in weight as moisture (matter volatile at 105–110°).

21

## LOSS ON IGNITION

Ignite 1 g of pigment in porcelain crucible over Meker burner to constant weight.<sup>2</sup>

22

## INSOLUBLE MATTER

Moisten 1 g of sample with few drops of alcohol, cover, add 40 ml of HCl (1+1), and boil gently 5–10 min. Wash off cover, evaporate to dryness, and heat at ca 150° for 30–60 min. to dehydrate residue. Moisten residue with 4 ml of HCl, allow to stand a few minutes, dilute with 100 ml of hot H<sub>2</sub>O, boil a few minutes, filter hot thru paper, and wash with hot H<sub>2</sub>O till washings give no test for Pb and Cl. Ignite paper and residue in Pt or porcelain crucible, cool, and weigh total insoluble matter.<sup>3</sup> (Insoluble matter may be filtered off on Gooch crucible, washed with hot H<sub>2</sub>O, dried at 105°, cooled, and weighed; then ignited, cooled, and weighed, when it is desired to get loss on ignition (combined H<sub>2</sub>O, organic matter, etc.), or when insoluble matter is not to be further examined. If sample contains Ti pigment, practically all the TiO<sub>2</sub> will be found in insoluble matter along with BaSO<sub>4</sub> and siliceous matter. Should an examination of insoluble matter be necessary, it is advisable to remove the TiO<sub>2</sub> before proceeding further. The TiO<sub>2</sub> may be removed (or determined on a separate portion) as directed under 19. After removing the TiO<sub>2</sub>, the residue containing siliceous matter and BaSO<sub>4</sub> may be ignited to remove filter.) To determine BaSO<sub>4</sub>, mix ignited insoluble matter with ca 10 times its weight of anhydrous Na<sub>2</sub>CO<sub>3</sub> (grinding mixture in agate mortar if necessary) and fuse in covered Pt crucible, heating ca 1 hour. Cool, and place crucible and cover in 200 ml glazed porcelain casserole (casserole is preferable to beaker as silica is dissolved from glass when in long contact with a strong Na<sub>2</sub>CO<sub>3</sub> soln). Add ca 100 ml of H<sub>2</sub>O and heat until mass is disintegrated. Filter on paper into 300 ml glazed porcelain casserole (leaving crucible and cover in original casserole) and wash casserole and filter thoroly with hot soln of Na<sub>2</sub>CO<sub>3</sub> (1%). Place casserole containing crucible and cover under funnel, pierce filter with glass rod, and wash residue into original casserole by means of jet of hot H<sub>2</sub>O. Wash paper with hot HCl (1+1) and then with hot H<sub>2</sub>O. Remove crucible and cover. Evaporate HCl soln to dryness, and heat at ca 150° 30–60 min. Moisten residue with ca 10 ml of HCl, dilute with 100 ml of hot H<sub>2</sub>O, boil a few minutes, filter hot thru the paper, and wash thoroly with hot H<sub>2</sub>O. Dilute filtrate to volume of 300 ml, bring to boiling and add, dropwise, 5 ml of H<sub>2</sub>SO<sub>4</sub> (1+4). Allow to stand in warm place ca an hour, filter on weighed Gooch crucible, wash with hot H<sub>2</sub>O, ignite, cool, and weigh as BaSO<sub>4</sub>. Subtract sum of percentages of BaSO<sub>4</sub> and TiO<sub>2</sub> from percentage of total insoluble matter and report result as percentage of insoluble siliceous matter.<sup>4</sup>

To determine silica, acidify filtrate from BaCO<sub>3</sub> filtration with HCl, boil to expel

<sup>1</sup> On an extracted and dried pigment, this determination is of little value. If original paint contained gypsum, a part of combined H<sub>2</sub>O of latter will be driven off in drying of extracted pigment and in "moisture" determination.

<sup>2</sup> This determination may serve as a rough or approximate check in many cases on the CO<sub>2</sub>, H<sub>2</sub>O, etc.

<sup>3</sup> See Ref. 1, under 13.

<sup>4</sup> Any soluble Al<sub>2</sub>O<sub>3</sub> (Fe<sub>2</sub>O<sub>3</sub>) and in most cases MgO, and sometimes some CaO, come from siliceous pigment used. MgO generally denotes presence of asbestine.



CO<sub>2</sub>, evaporate to dryness, bake to dehydrate silica, moisten with HCl, dilute with 100 ml of hot H<sub>2</sub>O, boil, and filter thru same paper used to recover silica from the BaCO<sub>3</sub> portion. Wash thoroughly with hot H<sub>2</sub>O and proceed as in silicate analysis.

If it is desired to determine Mg, combine this last filtrate with filtrate from final BaSO<sub>4</sub> separation and test for Al<sub>2</sub>O<sub>3</sub> and MgO in usual way. To recover MgO that may have dissolved in procedure for elimination of TiO<sub>2</sub>, make filtrate containing the TiO<sub>2</sub> just alkaline with NH<sub>4</sub>OH, bring to boiling, filter, and wash. Filtrate may be tested for MgO. Any Al<sub>2</sub>O<sub>3</sub> present will be precipitated along with the TiO<sub>2</sub>. To recover this, ignite and weigh as TiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub>. Deduct for TiO<sub>2</sub> present in sample; the difference is Al<sub>2</sub>O<sub>3</sub>.

## 23

## TOTAL LEAD AND ANTIMONY

Unite filtrate and washings (150–200 ml) from total insoluble matter, pass H<sub>2</sub>S into soln until it is saturated, add equal volume of H<sub>2</sub>O and again saturate with H<sub>2</sub>S. Filter, wash with H<sub>2</sub>O containing a little H<sub>2</sub>S and dissolve in hot HNO<sub>3</sub> (1+3), washing paper with hot H<sub>2</sub>O. Add 10–20 ml of H<sub>2</sub>SO<sub>4</sub> (1+1), evaporate until copious fumes of H<sub>2</sub>SO<sub>4</sub> are evolved, cool, and add ca 75 ml of H<sub>2</sub>O, and then ca 75 ml of 95% ethyl alcohol. Stir, let settle, filter on Gooch crucible, wash with dilute alcohol, and dry in oven at 105–110°; or, ignite gently in radiator<sup>1</sup> or muffle; cool, and weigh as PbSO<sub>4</sub>. Calculate to PbO.<sup>2</sup>

If pigment contains Sb, filter and wash sulfide precipitate as directed above; then wash precipitate with fine jet of H<sub>2</sub>O from the paper into porcelain dish or casserole, add 25 ml of NH<sub>4</sub> polysulfide, 1(i), cover vessel, and warm mixture at 40–60° 10–15 min. with frequent stirring. Wash off cover, filter thru paper used in first case, and wash with 2–3% Na<sub>2</sub>S or (NH<sub>4</sub>)<sub>2</sub>S soln. Discard filtrate. Dissolve residue in hot HNO<sub>3</sub> (1+3), and determine Pb as PbSO<sub>4</sub>, as described above. Or, original sulfide precipitate may be discarded and Pb determined on separate portion of pigment as follows: To 1 g of sample in covered beaker, add 40 ml of HCl (1+1) and boil gently 5–10 min. Wash off cover and evaporate to dryness. To residue add sufficient HCl to dissolve the PbSO<sub>4</sub> (with pigments containing considerable amounts of PbSO<sub>4</sub>, it may be necessary to add 15–20 ml of HCl), add ca 50 ml of hot H<sub>2</sub>O, boil a few minutes, filter hot thru paper, and wash with hot H<sub>2</sub>O until washings give no test for Pb. If sample contains no insoluble matter omit filtration. To filtrate add 20 ml of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84) and evaporate until dense white fumes of H<sub>2</sub>SO<sub>4</sub> are copiously evolved. Allow to cool, but not below 60°, and add slowly 50 ml of H<sub>2</sub>O while soln is agitated. Heat to boiling several minutes in order to insure complete soln of Sb sulfate. Allow the PbSO<sub>4</sub> to settle out until supernatant liquid is clear, not letting temp. fall below 60°. If liquid does not clear quickly heat it for longer period. When clear, pour soln thru weighed porcelain Gooch crucible with asbestos mat, decanting soln as completely as possible without allowing more than very small amount of PbSO<sub>4</sub> to go over into crucible. Add 10 ml more of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84) to the PbSO<sub>4</sub> in original beaker, and boil for several minutes. Cool, add slowly 30 ml of H<sub>2</sub>O, and again heat to boiling for a few minutes. Allow soln to cool to ca 60° and completely transfer the PbSO<sub>4</sub> to Gooch crucible. Wash with "lead acid," 1(j), to remove soluble sulfates and finally wash free of acid with dilute alcohol (equal parts of ethyl alcohol or denatured alcohol and H<sub>2</sub>O). Dry in oven at 105–110°, or ignite gently in radiator or muffle. Calculate to PbO, or determine as chromate as described below.

<sup>1</sup> U. S. Geological Survey Bull. 700 (1919), p. 33.

<sup>2</sup> It is not possible to determine amount of basic lead carbonate and lead sulfate when carbonates or soluble sulfates of other metals, such as Ca, are present. Neither basic lead carbonate nor basic lead sulfate is a definite compound.

If soluble compounds of Ba or Ca are present,  $\text{BaSO}_4$  and  $\text{CaSO}_4$  will be included with the  $\text{PbSO}_4$ . If soluble  $\text{SiO}_2$  is present, it will also be included with the  $\text{PbSO}_4$ . In such cases, the  $\text{PbSO}_4$  precipitate, after being washed with dilute alcohol, may be dissolved in acid  $\text{NH}_4$  acetate, 1(h), and the Pb determined as  $\text{PbCrO}_4$ , as directed below. For ordinary work, the amount of  $\text{BaSO}_4$  dissolved by the acetate treatment may be disregarded.

If pigment contains no soluble Sb, Ba, or Ca compounds, the Pb may be determined directly on original pigment, as follows: To 1 g of sample in covered beaker, add 25 ml of  $\text{HNO}_3$  (1+1), and boil gently a few minutes. Wash off cover, evaporate to dryness on steam bath, moisten with  $\text{HNO}_3$ , add hot  $\text{H}_2\text{O}$ , and heat a few minutes. Filter, and wash with hot  $\text{H}_2\text{O}$  until washings are Pb-free. Add 10–20 ml of  $\text{H}_2\text{SO}_4$  (1+1) to clear soln, evaporate, and determine Pb as  $\text{PbSO}_4$ , as above described.

In absence of soluble compounds of Sb, Fe, Al, and Ba, the following procedure may be used: Treat 1 g of original pigment with 25 ml of  $\text{HNO}_3$  (1+1) and proceed as above. To clear soln, diluted to 200 ml, add  $\text{NH}_4\text{OH}$  in slight excess, acidify with acetic acid, and add 4–6 ml more of this acid. Heat to boiling and add 10–15 ml of 10% soln of  $\text{K}_2\text{Cr}_2\text{O}_7$ . Heat until yellow precipitate assumes orange color and let settle. Filter on weighed Gooch crucible and wash by decantation until washings are colorless, finally transferring all precipitate. Wash with 95% alcohol and then with ether; dry to constant weight at  $110^\circ$ , cool, and weigh  $\text{PbCrO}_4$ . Calculate to PbO.

#### ANTIMONY OXIDE

24

##### *Method I. In ous condition*

Transfer 0.3 g of straight antimony oxide pigment, or 0.5 g of mixed pigment, to 500 ml Pyrex Erlenmeyer flask, and add 15 ml of  $\text{H}_2\text{O}$  and 25 ml of  $\text{HCl}$ . Cover with watch-glass, warm on steam bath 10–15 min. to dissolve the antimony oxide, wash off cover, and add 250 ml of  $\text{H}_2\text{O}$  and 15 ml of  $\text{H}_2\text{SO}_4$ . Boil 2 min., cool to  $10$ – $15^\circ$ , and titrate to faint pink tint with 0.1 N  $\text{KMnO}_4$  soln, 1(k). Calculate to  $\text{Sb}_2\text{O}_3$ .

25

##### *Method II. In ous and ic condition*

Transfer 0.3 g of straight  $\text{Sb}_2\text{O}_3$  pigment, or 0.5 g of mixed pigment, to 500 ml Pyrex Erlenmeyer flask, and add 15 ml of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84), 10 g of  $\text{K}_2\text{SO}_4$ , and 9 cm filter paper (to furnish C to act as reducing agent). Place funnel in neck of flask, and heat until soln becomes colorless. Cool, wash off funnel, dilute to 250 ml with  $\text{H}_2\text{O}$ , add 20 ml of  $\text{HCl}$ , and boil 2 min.; cool to  $10$ – $15^\circ$ , and titrate to faint pink tint with 0.1 N  $\text{KMnO}_4$  soln.<sup>1</sup>

26

##### *Method III. In presence of appreciable amounts of iron*

Treat 1 g of mixed pigment, or 0.3 g of straight  $\text{Sb}_2\text{O}_3$  pigment, in covered 250 ml beaker with 5 ml of  $\text{H}_2\text{O}$  and 20 ml of  $\text{HCl}$  (sp. gr. 1.19); heat on steam bath 15 min., cool, wash off cover, add 3 g of tartaric acid and 100 ml of hot  $\text{H}_2\text{O}$ , and digest a few minutes. Filter, catching filtrate in 500 ml Pyrex Erlenmeyer flask; wash thoroughly with hot  $\text{H}_2\text{O}$ , dilute to 300 ml with hot  $\text{H}_2\text{O}$ , and pass in  $\text{H}_2\text{S}$  until precipitation is complete. (If sample contains no insoluble matter, dissolve directly in 500 ml Pyrex Erlenmeyer flask, add tartaric acid, dilute, and pass in  $\text{H}_2\text{S}$ .) Filter, wash

<sup>1</sup> If digestion with  $\text{H}_2\text{SO}_4$  and  $\text{K}_2\text{SO}_4$  (plus filter paper) is continued after soln becomes colorless, some of antimony may be oxidized from the ous to the ic condition. In such cases, cool, wash off funnel, dilute to 100 ml with  $\text{H}_2\text{O}$ , add 1–2 g of  $\text{Na}_2\text{SO}_3$ , and boil until all  $\text{SO}_2$  is expelled. This is shown when no blue color is obtained with starch-iodate paper, 1(r); the volume will be reduced ca one-half. Dilute to 250 ml with  $\text{H}_2\text{O}$ , add 20 ml of  $\text{HCl}$  (sp. gr. 1.19), and boil 2 min.; cool to  $10$ – $15^\circ$ , and titrate to faint pink tint with 0.1 N  $\text{KMnO}_4$  soln. Calculate total Sb to  $\text{Sb}_2\text{O}_3$ . Subtract  $\text{Sb}_2\text{O}_3$  found by procedure given in first paragraph under 25 from total  $\text{Sb}_2\text{O}_3$  and calculate residual  $\text{Sb}_2\text{O}_3$  to  $\text{Sb}_2\text{O}_3$ .

with  $\text{H}_2\text{O}$  containing  $\text{H}_2\text{S}$  until free from  $\text{HCl}$ , return paper and precipitate to Erlenmeyer flask, add 15 ml of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84) and 10 g of  $\text{K}_2\text{SO}_4$ , place funnel in neck of flask, and heat until soln is colorless. Cool, wash off funnel, dilute to ca 250 ml with  $\text{H}_2\text{O}$ , add 20 ml of  $\text{HCl}$  (sp. gr. 1.19), boil 2 or 3 min., cool to ca  $10^\circ$ , and titrate to faint pink tint with 0.1 *N*  $\text{KMnO}_4$  soln, 1(k). Calculate total antimony to  $\text{Sb}_2\text{O}_3$ .<sup>1</sup>

27

## SOLUBLE BARIUM

Boil combined filtrate and washings, reduced in volume by evaporation if need be, from the  $\text{PbS}$  precipitate (total Pb) to expel  $\text{H}_2\text{S}$ . Add slight excess of  $\text{H}_2\text{SO}_4$  (1+4) over amount required to precipitate the Ba, heat to boiling, let stand on steam bath ca 1 hour, filter on weighed Gooch crucible, wash with hot  $\text{H}_2\text{O}$ , dry, ignite, cool, and weigh  $\text{BaSO}_4$ .<sup>2</sup> Calculate to BaO.

28

ALUMINA ( $\text{Fe}_2\text{O}_3$ ,  $\text{TiO}_2$ ,  $\text{P}_2\text{O}_5$ )

Boil filtrate from the  $\text{PbS}$  to expel  $\text{H}_2\text{S}$ , add few drops of  $\text{HNO}_3$ , and continue boiling a few minutes to oxidize any Fe that may be present. If soluble Ba was present, use filtrate from that determination. To soln containing at least 5 g of  $\text{NH}_4\text{Cl}$  per 200 ml of soln, or an equivalent amount of  $\text{HCl}$ , add a few drops of methyl red (0.2% alcoholic soln) and heat just to boiling. Carefully add  $\text{NH}_4\text{OH}$  (1+2) dropwise until color of soln changes to distinct yellow. Boil soln 1–2 min. and filter at once. Wash precipitate thoroly with hot 2%  $\text{NH}_4\text{Cl}$  soln.<sup>3</sup> Ignite precipitate, cool, and weigh as  $\text{Al}_2\text{O}_3$ .<sup>4</sup>

## TOTAL ZINC

29

*Method I*

To combined filtrate and washings from alumina precipitate, add sufficient  $\text{NH}_4\text{Cl}$  to give 5 g per 100 ml of soln, and then add 1 g of  $\text{NH}_4$  acetate.<sup>5</sup>

Render slightly acid with acetic acid and pass in current of  $\text{H}_2\text{S}$  to saturation. Allow precipitate to settle completely, filter on paper, and wash with 2% soln of acetic acid saturated with  $\text{H}_2\text{S}$ . Transfer precipitate and filter to vessel in which precipitation was effected, add 30 ml of  $\text{H}_2\text{O}$  and 10 ml of  $\text{HCl}$ , and heat until all Zn is in soln. Add 200 ml of  $\text{H}_2\text{O}$  and small piece of litmus paper. Add  $\text{NH}_4\text{OH}$  until slightly alkaline, and render just acid with  $\text{HCl}$ . Add 3 ml of  $\text{HCl}$ , heat nearly to boiling, and titrate with standard  $\text{K}_4\text{Fe}(\text{CN})_6$  soln as in standardizing that soln, 1(l).

30

*Method II*

Determine Zn directly on original sample as follows:<sup>6</sup> Weigh accurately ca 1 g (or amount that will give a buret reading approximately equal to that obtained in standardization) of pigment, and transfer to 400 ml beaker. Add 30 ml of  $\text{HCl}$  (1+2) and boil a few minutes. Add 200 ml of  $\text{H}_2\text{O}$  and small piece of litmus paper. Add  $\text{NH}_4\text{OH}$  until slightly alkaline and render just acid with  $\text{HCl}$ . Add 3 ml of  $\text{HCl}$ , heat nearly to boiling, and titrate with standard  $\text{K}_4\text{Fe}(\text{CN})_6$  soln as in standardizing that soln, 1(l).

<sup>1</sup> See Ref. 2 on p. 103, omitting last two sentences.

<sup>2</sup> This will include any  $\text{BaSO}_4$  that may have been dissolved as such. The weighed precipitate should be tested for  $\text{CaSO}_4$ , and if present, it should be removed by treating with hot dilute  $\text{HCl}$ , filtering, washing, igniting, and again weighing. Also see Ref. under 15.

<sup>3</sup> For very accurate work, or when precipitate is large, precipitate should be dissolved in  $\text{HCl}$  (1+1) and the precipitation repeated.

<sup>4</sup> This precipitate may also contain  $\text{Fe}_2\text{O}_3$ ,  $\text{TiO}_2$ , and  $\text{P}_2\text{O}_5$ .

<sup>5</sup> Gooch, *Representative Procedures in Quantitative Chemical Analysis*, 1st ed., p. 107.

<sup>6</sup> If sample contains Sb, it should be precipitated by  $\text{H}_2\text{S}$  in the hot acid soln, filtered off, washed, and filtrate neutralized, etc., for Zn. The  $\text{H}_2\text{S}$  precipitate may also contain  $\text{PbS}$ . If no sulfide separation is made, any Cd present will be counted as Zn.

31

*Method III*

When Fe is present, total Zn may be determined directly on original sample as follows:<sup>1</sup> Weigh accurately ca 1 g (or amount that will give a buret reading approximately equal to that obtained in standardization) of pigment, transfer to 250 ml beaker, moisten with alcohol, add 30 ml of HCl (1+2), and boil 2 or 3 min. Add ca 100 ml of H<sub>2</sub>O and ca 2 g of NH<sub>4</sub>Cl, make slightly alkaline with NH<sub>4</sub>OH, heat to boiling, and let settle on steam bath. Filter into 400 ml beaker, and wash residue once with hot H<sub>2</sub>O. Remove 400 ml beaker, pour HCl (1+2) on residue, catching filtrate therefrom in the 250 ml beaker, and wash a few times with hot H<sub>2</sub>O. Add to this filtrate 1 g of NH<sub>4</sub>Cl, make slightly alkaline with NH<sub>4</sub>OH, boil, let settle, filter on paper used for first filtration, and wash thoroly with hot H<sub>2</sub>O, catching filtrate and washings in the 400 ml beaker containing first filtrate. Add small piece of litmus paper, acidify with the HCl, add 3 ml of HCl, heat nearly to boiling, and titrate with standard K<sub>4</sub>Fe(CN)<sub>6</sub> as above.

32

*Method IV*

With pigments containing ZnO and ZnS, determine ZnO by weighing accurately 1 g of pigment and proceeding as directed under 18(b).

33

## SOLUBLE CALCIUM

Heat united filtrate and washings, reduced in volume if need be, from the ZnS precipitate, to boiling, and add 1 ml of NH<sub>4</sub>OH and an excess of hot saturated NH<sub>4</sub> oxalate soln. Continue boiling until precipitate becomes granular; let stand ca 1 hour, filter, and wash with hot H<sub>2</sub>O. Ignite, cool, and weigh as CaO;<sup>2,3</sup> or place beaker in which precipitation was made under funnel, pierce apex of filter with stirring rod, and wash precipitate into beaker with hot H<sub>2</sub>O, pouring warm H<sub>2</sub>SO<sub>4</sub> (1+4) thru paper and washing a few times. Add ca 30 ml of H<sub>2</sub>SO<sub>4</sub> (1+4), dilute to ca 250 ml, heat to 90°, and titrate at once with standard (0.1 N) KMnO<sub>4</sub> soln (temp. of soln should not be below 60° when end point is reached, *see* reagents). Calculate to CaO.<sup>4</sup> Fe value of KMnO<sub>4</sub> × 0.502 = CaO value.

34

## SOLUBLE MAGNESIUM

Acidify filtrate from the Ca precipitate with HCl, and add 10 ml of saturated soln of NaNH<sub>4</sub>HPO<sub>4</sub> and NH<sub>4</sub>OH dropwise, with constant stirring. When the crystalline MgNH<sub>4</sub>PO<sub>4</sub> has formed, add 5 ml excess of NH<sub>4</sub>OH. Allow soln to stand in cool place for not less than 4 hours, preferably overnight;<sup>5</sup> filter, and wash with H<sub>2</sub>O containing 2.5% of NH<sub>3</sub>. Dissolve precipitate in small quantity of hot HCl (1+1), dilute soln to ca 100 ml with H<sub>2</sub>O, add 1 ml of saturated soln of NaNH<sub>4</sub>HPO<sub>4</sub> and NH<sub>4</sub>OH dropwise, with constant stirring, until precipitate is again formed as described, and add 5 ml excess of NH<sub>4</sub>OH. Let precipitate stand in cool place for not less than 2 hours, filter on Gooch crucible, wash with H<sub>2</sub>O containing 2.5% of NH<sub>3</sub>, ignite, cool, and weigh as Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>.<sup>6</sup> Calculate to MgO.

35

## CARBON DIOXIDE

Fit a small Erlenmeyer flask with separator, CO<sub>2</sub>-free air inlet, and inclined con-

<sup>1</sup> See footnote 6, p. 104.

<sup>2</sup> Care must be exercised in this washing, as 1000 ml of boiling H<sub>2</sub>O will dissolve over 0.01 g of CaC<sub>2</sub>O<sub>4</sub>.

<sup>3</sup> For more accurate work, ignite the CaC<sub>2</sub>O<sub>4</sub> precipitate, cool, cautiously moisten with H<sub>2</sub>O, redissolve in HCl and dilute soln to 100 ml. Add NH<sub>4</sub>OH in slight excess, boil liquid, filter, and wash if precipitate appears. Then reprecipitate the Ca with NH<sub>4</sub>OH and (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub> as above, filter, wash, ignite, cool, and weigh; or, titrate as described. See also Ref. 1 under 15.

<sup>4</sup> See Ref. 1 under 13.

<sup>5</sup> The less the amount of Mg present, the longer the precipitate must be allowed to settle.

<sup>6</sup> If the sample contains Mn, it will be caught in large part with the Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>. If desired, Mn may be determined by dissolving the Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub> in HNO<sub>3</sub> and applying the bismuthate method.

denser connected to drying system— $\text{CaCl}_2$ , anhydrous Cu sulfate, then  $\text{CaCl}_2$  again. Connect to drying system absorption tubes filled with soda-lime followed by  $\text{CaCl}_2$  and observation bulbs containing  $\text{H}_2\text{SO}_4$  to show rate of gas flow.<sup>1</sup>

Place 1–2 g of pigment, depending upon probable  $\text{CO}_2$  content,<sup>2</sup> into Erlenmeyer flask and pour hot  $\text{H}_2\text{O}$  onto it. Connect to condenser and with weighed absorption tubes removed and observation bulbs attached directly to drying system, force current of  $\text{CO}_2$ -free air thru system until original air has been displaced. Close stopcock in separator, half fill with  $\text{HCl}$  (1+1), replace rubber stopper of funnel, insert absorption tubes between drying system and observation bulbs, and allow acid to flow into flask, slowly if there is much  $\text{CO}_2$ , rapidly if there is little. When effervescence diminishes in former case, at once in latter, light burner under flask and start flow of  $\text{H}_2\text{O}$  thru condenser. Keep flame low, so as to secure steady but quiet ebullition, and do not interrupt air current altho it should be reduced to slow rate. After elapse of sufficient time (usually 1 min.), extinguish flame and increase air current. When cool, disconnect soda-lime tubes and allow to stand in balance case until two weights taken 30 min. apart agree within 0.5 mg.

## 36

TOTAL SOLUBLE SULFUR COMPOUNDS<sup>3</sup>

Treat 1 g of pigment in 400 ml beaker with 10 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{HCl}$  saturated with Br, and 5 g of  $\text{NH}_4\text{Cl}$ . Digest (covered) on steam bath 5 min., dilute with hot  $\text{H}_2\text{O}$  to ca 200 ml, boil 5 min., filter to separate any insoluble matter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Nearly neutralize clear soln in covered beaker with  $\text{NaOH}$  soln, complete neutralization with dry  $\text{Na}_2\text{CO}_3$ , and add ca 2 g more of this reagent. Boil 10–15 min., wash off cover, let settle, filter, and wash with hot  $\text{H}_2\text{O}$ . Redissolve precipitate in  $\text{HCl}$  (1+1), reprecipitate with  $\text{Na}_2\text{CO}_3$  as above, filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Acidify united filtrates with  $\text{HCl}$ , adding ca 1 ml in excess. Boil to expel Br, and to clear boiling soln add slowly with stirring an excess of 10%  $\text{BaCl}_2$  soln. Let stand on steam bath at least 1 hour, filter on weighed Gooch crucible, wash thoroly with boiling  $\text{H}_2\text{O}$ , dry, ignite at dull red heat, cool, and weigh as  $\text{BaSO}_4$ . This will include soluble sulfates,  $\text{SO}_3$  formed from  $\text{SO}_2$ , and the  $\text{SO}_3$  that is formed from sulfide S.<sup>4</sup>

## 37

SOLUBLE SULFATE<sup>5</sup>

Treat 1 g of pigment with 10 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{HCl}$ , and 5 g of  $\text{NH}_4\text{Cl}$ . Boil until  $\text{H}_2\text{S}$  is expelled, adding more  $\text{HCl}$  (1+1) if necessary; dilute with hot  $\text{H}_2\text{O}$  to ca 200 ml, boil 5 min., filter to separate any insoluble matter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Nearly neutralize the clear soln with  $\text{NaOH}$  soln and make a double precipitation with  $\text{Na}_2\text{CO}_3$ , as in preceding method, finally weighing as  $\text{BaSO}_4$ , as described above.<sup>3</sup>

## 38

SULFIDE SULFUR<sup>6</sup>

Place 0.5–1 g of pigment in flask with ca 10 g of “feathered” or mossy Zn, add 50 ml of  $\text{H}_2\text{O}$ , and insert stopper carrying separator and exit tube. Run in 50 ml of  $\text{HCl}$  from funnel, having previously connected exit tube to 2 absorption flasks in series. The first flask contains 100 ml of alkaline  $\text{Pb}(\text{NO}_3)_2$  soln, 1(n), and the second

<sup>1</sup> Soda lime used must be porous, not hard and unabsorptive like that sometimes used for the combustion of nitrogenous organic substances.

<sup>2</sup> If the sample is high in sulfide, *e.g.*, contains a high percentage of lithopone, grind 1–2 g of the pigment with dry  $\text{K}_2\text{Cr}_2\text{O}_7$ , transfer to the evolution flask, add 50 ml of  $\text{H}_2\text{O}$  and run in  $\text{H}_2\text{SO}_4$  (1+1) from the separatory funnel. Or, place at the front of the purifying and drying train a tube containing acidified  $\text{CuSO}_4$  soln,  $\text{KMnO}_4$  soln, or  $\text{CrO}_3$  soln.

<sup>3</sup> See Ref. 1 under 13.

<sup>4</sup> See Ref. 1 under 27.

<sup>5</sup> See Ref. 1 under 13.

<sup>6</sup> Evolution Method of W. G. Scott, *White Paints and Painting Materials*, p. 257; see also Blair, *The Chemical Analysis of Iron*. The percentage of sulfide S can be calculated from percentages of total Zn and Zn soluble in 2–3% acetic acid, assuming the sulfide to be  $\text{ZnS}$ . See 32.

flask, 50 ml of the same soln as a safety device. After all acid has run into evolution flask, heat slowly, finally boiling until first appearance of steam in first absorption flask. Disconnect, let PbS settle, filter, and wash with cold  $H_2O$ , then with hot  $H_2O$ , till neutral to litmus paper and washings give no test for Pb. Dissolve PbS precipitate in hot  $HNO_3$  (1+3), and determine Pb as  $PbSO_4$ . Calculate to S. For very rapid work proceed as follows: Absorb the evolved  $H_2S$  in ammoniacal  $CdCl_2$  or  $ZnSO_4$  soln, 1(o), contained in 2 flasks connected in series, wash contents of absorption flasks into vessel with cold  $H_2O$ , dilute to ca 1 liter, acidify with  $HCl$ , and titrate with standard  $KIO_3$  soln, 1(p), using starch indicator, 1(q).

39

SULFUR DIOXIDE<sup>1</sup>

Transfer 10 g of pigment to suitable flask, insert stopper fitted with separator and spray trap delivery tube,<sup>2</sup> and attach latter to condenser. Place ca 150 ml of  $HCl$  (1+3) in funnel, stopcock being closed,<sup>3</sup> and connect other end of condenser with delivery tube that passes thru a 2-holed stopper and extends nearly to bottom of an absorption flask; thru other hole of stopper connect tube or flask to serve as safety device. Place 25 ml of 0.05 N I soln, 1(s), in absorption flask (dilute with  $H_2O$  if necessary) and 20 ml of 10% KI soln in safety tube and fit stopper in absorption flask. Open stopcock and allow acid to slowly enter flask. Before all acid is admitted, force air (washed with  $NaOH$  soln) thru top of separator (ca 2 bubbles per second in KI soln). Boil soln 3 min. with air passing thru, remove source of heat, and pass air thru 30 min. Disconnect absorption vessels, wash the KI soln into the I soln, and titrate at once with 0.05 N  $Na_2S_2O_3$  soln, using starch indicator. Run blank determination in exactly same manner except to omit pigment. Subtract this figure from previous one and calculate final result to  $SO_2$  (1 ml of 0.05 N I = 0.0016 g of  $SO_2$ ).

40

## MATTER SOLUBLE IN WATER

Transfer 2.5 g of pigment to graduated 250 ml flask, add 100 ml of  $H_2O$ , and boil 5 min. Cool to room temp., dilute to mark with  $H_2O$ , mix, and allow to settle. Filter supernatant liquid thru dry filter paper and discard first 20 ml of filtrate. Transfer 100 ml of clear filtrate to weighed dish, evaporate to dryness on steam bath, dry 1 hour in oven at 105–110°, cool, and weigh. Calculate percentage of water-soluble matter (nature of which may be determined by further examination as percentages of  $SO_3$  and  $CaO$  may be indicative).

41

## CALCULATIONS

Calculation of component pigments of a mixed or combination pigment may be difficult. Depending upon complexity of mixed pigment, certain assumptions must be made as to composition or formulas of component pigments and as to manner in which the acidic and basic radicals are combined. Add any  $Al_2O_3(Fe_2O_3)$  found in soluble portion to siliceous matter and report sum as "insoluble siliceous matter," unless the soluble Al is high. In this case, an aluminate is probably present, and the  $Al_2O_3$  should be reported as  $Al_2O_3$ . If small quantity of soluble Mg is found, add it to the siliceous matter. If soluble Mg is high, presence of  $MgCO_3$  is indicated, and the  $MgO$  is calculated to  $MgCO_3$  as pointed out below. The insoluble siliceous matter reported should be based on weight obtained on drying total insoluble matter at 105° if combined  $H_2O$  contained therein is to be considered.

<sup>1</sup> This method is not applicable in presence of sulfides decomposable under conditions given.

<sup>2</sup> A Knorr  $CO_2$  apparatus is convenient. The vertical condenser may be connected with absorption tower containing the I soln, followed by the KI soln in suitable tube.

<sup>3</sup> To minimize, if not eliminate, any possible oxidation by air, add ca 1 g (in one piece) of  $NaHCO_3$  to evolution flask, then add the acid directly to flask, omitting separator and current of air. Boil soln until ca 50 ml of distillate has passed over.

In the absence of ZnS or  $\text{TiO}_2$ , report  $\text{BaSO}_4$  as  $\text{BaSO}_4$ . If ZnS is present, calculate the  $\text{BaSO}_4$  equivalent by multiplying by 2.85; report sum of ZnS +  $\text{BaSO}_4$  as "lithopone." If  $\text{TiO}_2$  is present, calculate the  $\text{BaSO}_4$  equivalent by multiplying by 3.17; report sum of  $\text{TiO}_2$  +  $\text{BaSO}_4$  as "titanium pigment." Report residual  $\text{BaSO}_4$  as  $\text{BaSO}_4$ . If  $\text{TiO}_2$  is present and  $\text{BaSO}_4$  is absent or is present in smaller amount than would be indicated by above factor, then report  $\text{TiO}_2$  as  $\text{TiO}_2$ , and  $\text{BaSO}_4$  as  $\text{BaSO}_4$ . If  $\text{CaCO}_3$ ,  $\text{CaSO}_4$ ,  $\text{BaCO}_3$ , and  $\text{MgCO}_3$  are absent, calculate  $\text{CO}_2$  to basic carbonate white Pb,  $(\text{PbCO}_3)_2 \cdot \text{Pb}(\text{OH})_2$ , and soluble  $\text{SO}_3$  to  $\text{PbSO}_4$ . Any excess of Pb is calculated to PbO, added to the  $\text{PbSO}_4$ , and sum is reported as basic  $\text{PbSO}_4$ ; or, multiply the sum of  $\text{PbSO}_4$  + PbO by 0.058 to obtain the ZnO; add this result to the  $\text{PbSO}_4$  + PbO and report as basic sulfate white Pb. (The ZnO factor is based on assumption that average composition of commercial basic sulfate white Pb is: 78.5%  $\text{PbSO}_4$ , 16.0% PbO, and 5.5% ZnO.) PbO should not be reported except in presence of  $\text{PbSO}_4$ , unless entire analysis is reported in elementary or oxide form.

If sample contains  $\text{CO}_2$  but no soluble  $\text{SO}_3$ , calculate total Pb to basic carbonate white lead  $(\text{PbCO}_3)_2 \cdot \text{Pb}(\text{OH})_2$ ; calculate residual  $\text{CO}_2$  to  $\text{CaCO}_3$ , then to  $\text{BaCO}_3$  and  $\text{MgCO}_3$  if soluble Ba and Mg are present in sufficient amounts to indicate presence of these carbonates. (The  $\text{CO}_2$  result will be index.) Any residual CaO is probably from siliceous matter and should be added to insoluble siliceous matter.

Any small amount of soluble Ba may be from the  $\text{CaCO}_3$  used or may be due to solubility of  $\text{BaSO}_4$  if this compound is present in original pigment. This Ba may be calculated to  $\text{BaSO}_4$ , and added to  $\text{BaSO}_4$  found in insoluble matter.

If sample contains soluble  $\text{SO}_3$  but no  $\text{CO}_2$ , calculate CaO to  $\text{CaSO}_4$  or  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ; residual  $\text{SO}_3$  to  $\text{PbSO}_4$ ; add residual PbO to  $\text{PbSO}_4$  and report sum as basic  $\text{PbSO}_4$ ; or, multiply  $\text{PbSO}_4$  + PbO by 0.058 and add result to the  $\text{PbSO}_4$  + PbO, and report total as basic sulfate white Pb.

If sample contains  $\text{CaCO}_3$  ( $\text{MgCO}_3$ ,  $\text{BaCO}_3$ ) and also basic sulfate white Pb, or  $\text{CaSO}_4$  and basic carbonate white Pb, or a mixture of these, it is not possible to determine or calculate amount of  $\text{PbCO}_3$  or  $\text{PbSO}_4$  with any degree of certainty.<sup>1</sup> Appreciable amounts of CaO and  $\text{SO}_3$  in water-soluble matter indicate probable presence of  $\text{CaSO}_4$  in original pigment. The following arbitrary calculations may be made: calculate water-soluble  $\text{SO}_3$  to  $\text{CaSO}_4$  or  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , subtract this  $\text{SO}_3$  from total soluble  $\text{SO}_3$  and calculate remainder to  $\text{PbSO}_4$ ; calculate residual CaO to  $\text{CaCO}_3$ , and then residual  $\text{CO}_2$  to  $(\text{PbCO}_3)_2 \cdot \text{Pb}(\text{OH})_2$ . If there is excess of  $\text{CO}_2$ , calculate to  $\text{MgCO}_3$  or  $\text{BaCO}_3$ , if amounts of soluble Mg and Ba indicate probable presence of these carbonates. Add residual PbO to  $\text{PbSO}_4$  and calculate, as above, to basic sulfate white Pb. The procedure followed by Federal Specifications Board should be noted.<sup>2</sup>

Report total Sb as  $\text{Sb}_2\text{O}_3$ .

Calculate sulfide S to ZnS, subtract Zn equivalent to S from total Zn, then subtract Zn required for basic sulfate white Pb, and report remainder as ZnO.

Report moisture loss on ignition,  $\text{SO}_2$ , and matter soluble in  $\text{H}_2\text{O}$  directly.

<sup>1</sup> See Ref. 1 under 27 and Ref. 1 under 13.

<sup>2</sup> Federal Specifications Board Specification TT-P-36 for Paints: "The total Pb dissolved in acetic acid (1+6) and hot  $\text{NH}_4$  acetate, weighed as  $\text{PbSO}_4$ , and this weight multiplied by factor 0.883 shall be considered white lead. (It is not possible to determine amount of  $\text{PbCO}_3$  and  $\text{PbSO}_4$  when carbonates or sulfates of other metals, such as Ca, are present. Also neither basic  $\text{PbCO}_3$  nor basic  $\text{PbSO}_4$  is a definite compound. The factor to convert  $\text{PbSO}_4$  to  $(\text{PbCO}_3)_2 \cdot \text{Pb}(\text{OH})_2$  is 0.854, to convert  $\text{PbSO}_4$  to  $\text{PbSO}_4 \cdot \text{PbO}$  is 0.868, and to convert  $\text{PbSO}_4$  to  $(\text{PbSO}_4)_2 \cdot \text{PbO}$  is 0.913. The arbitrary factor used under this specification is the mean of largest and smallest of these 3 factors.)"

OLEO-RESINOUS VARNISHES<sup>1</sup>—OFFICIAL

42

## PRELIMINARY PROCEDURE

Make all tests at 21–32° (70–90°F.) and in diffused light (not in direct sunlight). After opening can of varnish and using part of contents, place remainder immediately in air-tight containers which varnish practically fills, leaving not more than 2% of air space.

43

## APPEARANCE

Pour some of thoroly mixed sample into clear glass bottle or test tube, 1.5–2.0 cm in diameter, to depth of at least 2.5 cm. Examine varnish by transmitted light to see if it is clear and transparent.

44

## COLOR

Prepare standard color solns by dissolving 1, 2, 3, 4, 5, and 6 g, respectively, of pure powdered  $K_2Cr_2O_7$  in 100 ml of  $H_2SO_4$ , applying gentle heat, if necessary, to effect soln of the  $K_2Cr_2O_7$ . (Since the  $K_2Cr_2O_7$ – $H_2SO_4$  solns must be freshly made for this color comparison, it is frequently more convenient to compare samples with a series of permanently sealed tubes of varnish which have been previously found to be lighter in color than the standard solns. Stabilized caramel solns or other permanent colored liquids may be used as secondary standards.)

Pour each of standard color solns and a sample of the varnish to be tested into thin-walled glass tubes 1.5–2.0 cm in diameter to depth of at least 2.5 cm. Make color comparison by placing tubes close together and looking thru them by transmitted light. If permanently sealed tubes of varnish are used for the color comparison and the sample of the varnish being tested is found to be darker than a standard tube of varnish, make final comparison with freshly prepared  $K_2Cr_2O_7$ – $H_2SO_4$  solns. State color of varnish in terms of the standard (calling the standards No. 1, No. 2, No. 3, etc.) which it is equal to or lighter than sample.

45

## NON-VOLATILE MATTER

Pour portion of sample of varnish into stoppered bottle or weighing pipet and weigh. Transfer ca 1.5 g of sample to weighed, flat-bottomed metal dish ca 8 cm in diameter (a friction-top can plug is satisfactory). Reweigh container and calculate by difference exact weight of the portion of sample transferred to weighed dish. Heat dish with contents 3 hours in oven maintained at 105–110° and weigh after cooling.

Take ratio of weight of residue to that of sample, expressed in percentage, as percentage of non-volatile matter in varnish.

46

## ELASTICITY OR TOUGHNESS

Proportionately reduce elasticity or toughness of varnish by addition of a standard soln of kauri gum in pure spirits of turpentine, 47, or proportionately increase elasticity or toughness of varnish by means of addition of linseed oil, 48.

47

*Addition of Kauri Gum to Reduce Elasticity*

*Standard kauri gum soln.*—Arrange on balance a distillation flask, water-cooled condenser, and tared receiver. Place clear, bright, hard pieces of kauri gum broken to size of a pea in flask to ca one-third its capacity. Carefully melt and distil gum

<sup>1</sup> Standard Methods (D 154–28) of American Society for Testing Materials edited to conform in part to the A.O.A.C. style. A.S.T.M. Standards, 1933, Pt. II, p. 698.



until 25% by weight is collected in tared receiver, at which time of distillation the thermometer in distillation flask, with bulb at level of discharging end of flask, should register ca 316°. Pour residue into clean pan and when it is cold break into small pieces. Place in carefully tared beaker quantity of the small broken pieces of kauri gum, together with twice its weight of freshly redistilled spirits of turpentine, using only that portion distilling between 153 and 170°, and dissolve by heating to temp. of ca 149°. When cooled, bring mixture to correct weight by addition of quantity of redistilled spirits of turpentine necessary to replace loss by evaporation during dissolving of gum.

Cut test panels from bright tin plate weighing not more than 25 g nor less than 19 g per sq dm (0.51–0.39 lb per sq ft). (It is important that the tin plate be within limits prescribed. Commercial. No. 31 gage, bright tin plate should weigh ca 0.44 lb per sq ft.) Use a panel ca 7.5 by 13 cm and thoroly clean with benzene immediately before using. (It is important to have rags used in wiping panels clean.)

Having carefully determined non-volatile content of varnish according to 45, take 100 parts of the varnish by weight, add quantity of the standard kauri gum soln equivalent to 50% by weight of the non-volatile matter in the varnish, and mix thoroly. (20 g sample of varnish should be sufficient for each reduction.) If non-volatile content of the varnish is 48.6%, add 4.86 g of standard kauri gum soln to the 20 g of varnish to obtain 50% reduction. (50% standard kauri gum reduction is given to illustrate method. Any other percentage of standard kauri gum reduction may be used, depending on sample being tested.)

Flow varnish upon one of tin panels and stand panel in nearly vertical position at room temp. for nearly 1 hour. Place panel in horizontal position in properly ventilated oven and bake 5 hours at 95–100°. Remove panel from oven and permit it to cool at room temp. (preferably 24°) 15 min.

Place panel with varnished side uppermost over 3 mm rod, held firmly by suitable supports, at a point equidistant from top and bottom edges of panel and bend double rapidly. The varnish should show no cracking whatsoever at point of bending. For accurate results always bend panel at 24°, as a lowering of temp. will lower percentage of reduction that varnish will stand without cracking, while an increase in temp. will increase this percentage.

Report varnishes that do not show cracks under this test as passing a 50% reduction, and report those that do crack as not passing a 50% reduction.

Re-test the varnishes that have not cracked, changing amount of reduction to 60%; if they pass this percentage, test again with a 70% reduction. In a similar manner re-test varnishes that have cracked at 50%, using reductions of 40 and 30%. In this way determine limits within 10% at which a varnish passes one percentage of reduction and does not pass the next. For example, varnishes may be reported as passing 40%, and breaking at 50%.

#### 48

#### *Addition of Linseed Oil to Increase Elasticity*

(Applicable to varnishes less elastic or less tough than zero kauri reduction.)

Follow procedure described in 47, except to replace the 33 $\frac{1}{3}$ % soln of kauri gum in turpentine by 66 $\frac{2}{3}$ % soln of heat-bodied linseed oil in order to proportionately increase elasticity of varnish under test.

*Standard bodied oil soln.*—Heat a high grade of alkali-refined linseed oil of acid number less than 1.0 in an open kettle at 300°  $\pm$  5° until viscosity of oil after cooling is between 6 and 10 poises at 25°. Standardize a quantity of the heat-bodied linseed oil by reducing it with one-half its weight of pure redistilled turpentine, using only that portion of turpentine distilling between 153 and 170°.

Conduct addition of standard bodied oil soln to varnish, flowing on, baking, and bending of test panel exactly as in 47.

In reporting results, give minimum percentage of oil soln that must be added to varnish, based on its non-volatile content, so that final mixture when flowed on a test panel and baked does not crack on subsequent bending over a 3 mm rod.

## 49

FLASH POINT<sup>1</sup>

Use the "Tag" closed tester and make test in dim light so that flash may be seen plainly. Surround tester on three sides with inclosure to prevent draughts. (A shield ca 18" square and 2' high, open in front, is satisfactory, but any safe precaution against possible room draughts is acceptable. Tests made in laboratory hood or near ventilators will give unreliable results.) See that tester is firm and level. (For accuracy, flash-point thermometers especially designed for the instrument should be used, as position of bulb of thermometer in sample cup is important.)

Put water-bath thermometer in place, and put receptacle under over-flow spout. Fill bath with H<sub>2</sub>O at such temp. that, when testing is started, it will be at least 20°F. below probable flash point of sample to be tested.

Put sample cup in place in water bath. Measure 50 ml of sample to be tested in pipet or graduate, and place in sample cup. Have temp. of sample at least 20°F. below its probable flash point when testing is started. Destroy any bubbles on surface of sample. Put on cover with flash-point thermometer in place and with  $\frac{1}{8}$ " rubber tube connecting gas supply pipe to gas connection on cover. Light pilot light on cover and adjust flame to size of small bead on cover.

Light and place heating lamp, filled with alcohol, in base of tester and see that it is centrally located. Adjust flame of the alcohol lamp so that temp. of sample in cup rises at rate of ca 1.8°F. per min. (not faster than 2°F. nor slower than 1.6°F. per min.).

Record barometric pressure which, in absence of laboratory instrument, may be obtained from nearest Weather Bureau Station. Also record temp. of sample at start.

When temp. of sample reaches 9°F. below probable flash point of sample, turn knob on cover so as to introduce test flame into cup, and promptly turn it back again. Do not let it snap back. (Time consumed in turning knob down and back should be ca 1 full second, or time required to pronounce distinctly words "one-thousand-and-one." Record time and temp. of sample when making first introduction of test flame.

Repeat application of test flame at every 1°F. rise in temp. of sample until there is a flash within cup. Do not be misled by an enlargement of test flame or halo around it when it enters cup, or by slight flickering of flame; the true flash consumes the gas in top of cup and causes a very slight explosion. Record time at which flash point is reached and also record flash point.

If rise in temp. of sample, from time of making first introduction of test flame to that at which flash point is reached, was faster than 2°F. or slower than 1.6°F. per min., question test and adjust alcohol heating lamp to correct rate of heating. (It will be found that wick of this lamp can be so accurately adjusted as to give a uniform rate of rise in temp. within above limits.

Having completed preliminary test, remove heating lamp, lift up cover, and wipe off thermometer bulb. Lift out cup, and empty and carefully wipe it. Throw away all samples that have been used once in making a test.

Pour cold H<sub>2</sub>O into water bath, allowing it to overflow into receptacle, until

<sup>1</sup> Standard Method (D 56) of the A.S.T.M. edited to conform to the A.O.A.C. style. A.S.T.M. Standards, 1933, Pt. II, p. 663.

temp. of  $H_2O$  in bath is lowered to  $15^\circ F.$  below flash point of sample, as shown by previous test.

Place cup in bath and measure into it 50 ml charge of fresh sample. Proceed to repeat test as described above, introducing test flame for first time at temp. of  $10^\circ F.$  below flash point obtained on previous test.

If two or more determinations agree within  $1^\circ F.$  consider average of these results, corrected for barometric pressure, the flash point. If two determinations do not check within  $1^\circ F.$ , make third determination and, if maximum variation of three tests is not greater than  $2^\circ F.$ , consider their average, after correcting for barometric pressure, the flash point.

Make correction for barometric pressure only in cases of dispute or when the barometer reading varies more than  $\frac{1}{2}$ " (13 mm) from standard pressure of 29.92" (760 mm). When barometer reading is below this standard pressure, add to thermometer reading  $1.6^\circ F.$  for each inch (25 mm) of barometric difference to obtain true flash point. When barometer reading is above standard pressure, deduct  $1.6^\circ F.$  for each inch (25 mm) of barometric difference to obtain true flash point.

50

## VISCOSITY

Determine viscosity by comparison at  $25^\circ$  with secondary standards (in bubble tubes) whose viscosity expressed in poises has been accurately determined at that temp.<sup>1</sup>

51

## WATER TEST

Pour the varnish on one of tin panels described in 47, allow to drain in nearly vertical position, and dry 48 hours. Place panel in beaker containing  $H_2O$  at  $21$ – $32^\circ$  to depth of ca 7 cm, immersing end of panel that was uppermost during drying, and allow to remain 18 hours. Remove panel from  $H_2O$ , wipe carefully, and allow to dry out at  $21$ – $32^\circ$ . Note time required for whitening, if any, to disappear. Report results as follows:

- (a) Not visibly affected.
- (b) Whitening disappears within 20 min.
- (c) Whitening does not disappear in 20 min., but does disappear within 2 hours.
- (d) Whitening does not disappear within 2 hours, but does disappear within 24 hours.
- (e) Whitening does not disappear within 24 hours.

Blooming, which sometimes occurs on immersion, is considered a degree of whitening.

RAW LINSEED OIL<sup>2</sup>—OFFICIAL

52

## PREPARATION OF SAMPLE

Thoroughly agitate sample before removing portions for analysis.

53

SPECIFIC GRAVITY (APPARENT)  
( $15.5^\circ/15.5^\circ$ )

Use a pycnometer, accurately standardized and having capacity of at least 25 ml, or any other equally accurate method, making test at  $15.5^\circ$ ,  $H_2O$  being 1.000 at  $15.5^\circ$ .

<sup>1</sup> Circ. 178, Scientific Section, Paint Manufacturers' Association of United States.

<sup>2</sup> A.S.T.M. Standard Specifications for Raw Linseed Oil (D 234-28), A.S.T.M. Standards, 1933, Pt. II, pp. 644-649.

## IODINE ABSORPTION NUMBER

54

*Wijs Method*

Proceed as directed in 10, using 0.09–0.15 g of oil.

## SAPONIFICATION NUMBER

55

## REAGENT

*Sulfuric acid soln.*—0.5 *N*. Add ca 15 ml of  $\text{H}_2\text{SO}_4$  to  $\text{H}_2\text{O}$ , cool, and dilute to 1000 ml. Determine exact concentration by titrating against freshly standardized NaOH or by any other accurate method. Either adjust to exactly 0.5 *N* strength or leave as originally made, applying appropriate correction.

56

## DETERMINATION

Weigh ca 2 g of oil in 300 ml Erlenmeyer flask. Add 25 ml of alcoholic NaOH or KOH soln, 1(c). Put condenser loop inside neck of flask and heat on steam bath 1 hour. Cool, add phenolphthalein as indicator, and titrate with 0.5 *N*  $\text{H}_2\text{SO}_4$ . Run two blanks with alcoholic NaOH soln, 1(c). These should check within 0.1 ml. From difference between number of ml of 0.5 *N*  $\text{H}_2\text{SO}_4$  required for blank and for determination, calculate saponification number (mg KOH required for 1 g of oil).

57

## UNSAPONIFIABLE MATTER

Weigh 8–10 g of oil and transfer to 250 ml long-necked flask. Add 5 ml of NaOH (1+1) and 50 ml of 95% ethyl alcohol. Put condenser loop inside neck of flask and boil 2 hours. Occasionally agitate flask to break up liquid, but do not project liquid onto sides of flask. At end of 2 hours, remove condenser and allow liquid to boil down to ca 25 ml.

Transfer mixture to 500 ml glass-stoppered separator, rinsing with  $\text{H}_2\text{O}$ . Dilute with  $\text{H}_2\text{O}$  to 250 ml, add 100 ml of redistilled ether, stopper, and shake 1 min. Let stand until the two layers separate sharp and clear. Draw off all but 1 or 2 drops of aqueous layer into second 500 ml separator and repeat process, using 60 ml of ether. After thoro separation, draw off aqueous layer into 400 ml beaker, then the ether soln into first separator, rinsing down with a little  $\text{H}_2\text{O}$ . Return aqueous soln to second separator and shake out again with 60 ml of ether in similar manner, finally drawing aqueous soln into beaker and rinsing ether into first separator. Shake combined ether soln with combined  $\text{H}_2\text{O}$  rinsings and let layers separate sharp and clear. Draw off  $\text{H}_2\text{O}$  and add it to main aqueous soln. Shake ether soln with two portions of  $\text{H}_2\text{O}$  (ca 25 ml each). Add these to main  $\text{H}_2\text{O}$  soln.

Swirl separator so as to bring last drops of  $\text{H}_2\text{O}$  down to stopcock and draw off until ether soln just fills bore of stopcock. Wipe out stem of separator with a bit of cotton on a wire. Draw ether soln (portionwise if necessary) into 250 ml flask and distil off. While still hot drain flask into small weighed beaker, rinsing with a little ether. Evaporate this ether, cool beaker, and weigh. As the unsaponifiable oil from adulterated drying oils may be volatile and as a consequence may evaporate on long heating, heat beaker on warm plate, occasionally blowing out with current of dry air. Discontinue heating as soon as odor of ether is gone.

## ACID NUMBER

58

## REAGENT

*Standard sodium hydroxide soln.*—Prepare a stock concentrated soln by dissolving NaOH in  $\text{H}_2\text{O}$  in proportion of 200 g of NaOH to 200 ml of  $\text{H}_2\text{O}$ . Allow this soln to

cool and settle in a stoppered bottle for several days. Decant clear liquid from precipitate of  $\text{Na}_2\text{CO}_3$  into another clean bottle. Add clear  $\text{Ba}(\text{OH})_2$  soln until no further precipitate forms. Again allow to settle until clear. Draw off ca 175 ml and dilute to 10 liters with freshly boiled  $\text{H}_2\text{O}$ . Preserve in stock bottle provided with large guard tube filled with soda lime. Determine exact strength by titrating against pure benzoic acid, using phenolphthalein as indicator. This soln will be ca 0.25 *N*, but do not attempt to adjust it to any exact value. Determine its exact conc. and make proper corrections in using it.

59

## DETERMINATION

Weigh 5–10 g of oil and transfer to 300 ml Erlenmeyer flask. Add 50 ml of a mixture of equal parts by volume of alcohol and reagent benzene, previously titrated to very faint pink with dilute alkali soln, using phenolphthalein as indicator. Add phenolphthalein indicator and titrate at once to faint permanent pink color with the  $\text{NaOH}$  soln. Calculate acid number (mg  $\text{KOH}$  per g of oil).

## FOOTS (PER CENT)

60

## REAGENTS

(a) *Acid calcium chloride soln.*—Saturate with  $\text{CaCl}_2$  a mixture of 90 parts of  $\text{H}_2\text{O}$  and 10 parts of  $\text{HCl}$ .

(b) *Acetone.*—C. P. or A.S.T.M.D. 329.

61

## DETERMINATION

With all materials at 20–27°, mix by shaking exactly 1 min. in graduated tube, 25 ml of the well-shaken sample of oil, 25 ml of acetone, and 10 ml of the acid  $\text{CaCl}_2$  soln. Clamp tube in vertical position and allow mixture to settle 24 hours, keeping temp. 20–27°. Determine volume of stratum lying between the clear  $\text{CaCl}_2$  soln and the clear acetone and oil mixture to nearest 0.1 ml or fraction thereof. This volume  $\times 4$  = amount of foots present as percentage by volume.

(The graduated tube may be buret or color comparison tube. It should have an internal diameter of 1.0–1.5 cm, and a capacity of not less than 70 ml. The graduations in 0.1 ml should extend at least 10–50 ml above bottom of tube.)

(a) *Heated oil test.*—Heat portion of oil to 65°, hold it within 2° of that temp. 10 min., then cool to room temp. (20–27°). Subject sample promptly to foots test as directed above.

(b) *Chilled oil test.*—Heat portion of sample to 65°, hold it within 2° of that temp. 10 min., place in clean dry bottle, stopper tightly, and place in cracked ice and  $\text{H}_2\text{O}$  mixture (0°) exactly 2 hours. Keep bottle exactly 30 min. in water bath at 25°, then subject promptly to foots test as directed above.

62

## LOSS ON HEATING AT 105–110°

Weigh 10 g of the oil in accurately weighed 50 ml Erlenmeyer flask. Heat in oven at 105–110° 30 min., while passing  $\text{CO}_2$  gas thru oven. Cool in current of  $\text{CO}_2$  gas and weigh. Calculate percentage loss.

63

## COLOR

Prepare fresh soln of 1 g of pure  $\text{K}_2\text{Cr}_2\text{O}_7$  in 100 ml of  $\text{H}_2\text{SO}_4$ . Place oil and  $\text{K}_2\text{Cr}_2\text{O}_7$  soln in separate thin-walled, clear-glass tubes of same diameter (1–2 cm) to depth of not less than 2.5 cm, and compare depths of color by looking trans-

versely thru columns of liquid by transmitted light. State whether or not oil is darker than the  $K_2Cr_2O_7$  soln.

### BOILED LINSEED OIL<sup>1</sup>—OFFICIAL

64 PREPARATION OF SAMPLE.—*See* 52.

65 SPECIFIC GRAVITY (APPARENT).—*See* 53.

#### IODINE ABSORPTION NUMBER

66 *Wijs Method*

Proceed as directed under 10, using 0.09–0.15 g of oil.

67 SAPONIFICATION NUMBER.—*See* 55.

68 UNSAPONIFIABLE MATTER (PER CENT).—*See* 57.

69 ACID NUMBER.—*See* 58.

70 LOSS ON HEATING AT 105–110°.—*See* 62.

71 TIME OF DRYING ON GLASS

Flow sample over perfectly clean glass plate and place plate in vertical position in air at  $30^\circ \pm 2^\circ$  and humidity of  $32\% \pm 4\%$  saturation. After ca 2 hours, test film at intervals with finger at points not less than 2.5 cm from edges. Film shall be considered dry when it no longer adheres to finger and does not rub up appreciably when finger is lightly rubbed across surface.

72 ASH

Weigh carefully in weighed porcelain crucible or dish 10–25 ml of sample and place on stone slab on floor of hood. Ignite by playing flame of burner on surface of oil and allow to burn quietly until most of oil is burned off; transfer to muffle or over flame and continue heating at low temp. (not over a dull red) until all carbonaceous matter is consumed. Cool, weigh, and compute percentage of ash.

73 LEAD

Dissolve ash obtained as directed under 72 in  $HNO_3$  (1+10), to which a little  $H_2O_2$  has been added, and determine Pb by the sulfate or any other equally accurate method.

74 APPEARANCE

Transfer a portion of sample to clear glass tube and note appearance. Report on clarity or presence of sediment.

---

<sup>1</sup> A.S.T.M. Standard Specifications for Boiled Linseed Oil (D 260–33), A.S.T.M. Standards, 1933, Pt. II, pp. 638–643.

## X. LEATHERS—TENTATIVE

### VEGETABLE TANNED LEATHER

1

#### PREPARATION OF SAMPLE<sup>1</sup>

Reduce leather by cutting, planing, sawing, shredding, grinding, or rasping to as fine a state of subdivision as is practicable. Avoid heating sample during preparation and especially do not use unsuitable grinding mills that cause heating. Mix thoroly and place in tightly covered containers.

#### MOISTURE<sup>2</sup>

2

##### *Method I*

Place 5–10 g of prepared sample, 1, in tared, wide, shallow weighing bottle (or similar dish, which can be covered tightly), and dry in electric oven 15 hours at 100–102°. Cover weighing bottle, cool in desiccator containing H<sub>2</sub>SO<sub>4</sub>, and weigh. The moisture in leather as received may be determined by quickly cutting a representative portion of sample into small pieces and drying as directed without further preparation.

##### *Method II—By Toluene Distillation*

3

#### APPARATUS

- (a) *500 ml flask*.—Erlenmeyer, or distilling flask of Pyrex or other resistant glass.
- (b) *Receiving tube*.—Graduated in tenths of a ml.
- (c) *Liebig condenser*.—Sealed-in, straight-tube, ca 25 cm (10") long, with delivery tube ca 9.5 mm (0.375") in diameter.

Assemble apparatus as shown in XXVII, 3. Before each distillation clean condenser and receiving tube with CrO<sub>3</sub>-H<sub>2</sub>SO<sub>4</sub> mixture; rinse thoroly with H<sub>2</sub>O, then with alcohol; and dry in oven or with current of air. Calibrate receiving tube by distilling toluene containing known quantities of H<sub>2</sub>O. Read volume of H<sub>2</sub>O to 0.01 ml.

4

#### DETERMINATION

Weigh 20 g of prepared sample, 1, and transfer to distilling flask. Immediately add ca 200 ml of dry toluene having a b.p., under normal pressure, of 110–112°, and connect flask with receiving tube and condenser. Fill receiving tube with toluene, pouring it thru condenser. Heat distilling flask gently and distil at rate of ca 4 drops per second exactly 2 hours. At end of 1, 1.25, 1.5, 1.75, and 2 hours' distillation, wash down condenser by pouring toluene in at top while brushing thoroly with tight-haired, close-fitting tube brush that has been boiled previously in toluene. (A long handle may be made by fastening to brush a piece of heavy Cu wire.) At end of 2 hours, disconnect receiving tube, dislodge any drops of H<sub>2</sub>O on inside by rubbing with a piece of light Cu wire twisted at one end into a loop, and allow tube to come to room temp. Read volume of H<sub>2</sub>O to 0.01 ml and make such calibration correction as may be necessary. Assuming that 1 ml of H<sub>2</sub>O weighs 1 g, calculate percentage of moisture.

5

#### TOTAL ASH<sup>3</sup>

Incinerate slowly 5 g of prepared sample, 1, at maximum of ca 600°. If difficulty is experienced in burning off C, leach residue with hot H<sub>2</sub>O, filter on ashless filter,

dry, and ignite filter and residue. Add filtrate, evaporate to dryness, and ignite. Cool in desiccator containing  $\text{H}_2\text{SO}_4$  and weigh.

The ash may be examined for acids and bases by any suitable method. Fe, Al, Mg,<sup>4</sup> Na, Ba, Ca, and Pb are the bases, and HCl and  $\text{H}_2\text{SO}_4$  are the acids which it may be necessary to determine.

6

INSOLUBLE ASH<sup>5</sup>

Quantitatively remove leather remaining after extraction of water-soluble material as directed under 9, dry at temp. not exceeding  $60^\circ$ , weigh, and slowly incinerate a portion equal to exactly  $\frac{1}{3}$  of total weight until all C is burned off. Cool in desiccator containing  $\text{H}_2\text{SO}_4$  and weigh. Calculate insoluble ash on basis of original leather represented.

7

PETROLEUM BENZIN EXTRACT<sup>6</sup>

Place 5 g of prepared leather, 1, in fat-free paper thimble, cover with layer of fat-free cotton, and extract in Johnson or Soxhlet extractor 8–10 hours with petroleum benzin distilling at  $50\text{--}80^\circ$ . (Heavily greased leathers, containing 15% or more fat, will require maximum time.) Remove receiving flask, evaporate petroleum benzin on steam bath, and dry residue at  $98\text{--}100^\circ$  for periods of  $\frac{1}{2}$  hour each until practically constant weight is obtained. Avoid prolonged continuous heating, resulting possibly in partial volatilization or oxidation of extract.

MINERAL ACIDITY<sup>6</sup>

8

*Modified Proctor-Searle Method<sup>7</sup>*

Weigh 2 g of prepared leather, 1, into Pt dish; add 40 ml of 0.1 N  $\text{Na}_2\text{CO}_3$  soln, mix thoroly, and evaporate to complete dryness on steam bath. Place residue in electric muffle furnace at  $20\text{--}30^\circ$ , slowly raise temp. of muffle to  $600^\circ \pm 10^\circ$  in 2 hours and maintain at this temp. an additional hour. Remove dish, allow to cool, and carefully moisten residue with hot  $\text{H}_2\text{O}$ , adding ca 25 ml, and break up lumps with glass rod. Filter into 300 ml flask thru an ashless paper and wash 4 or 5 times with hot  $\text{H}_2\text{O}$ . Return filter paper and residue to its dish, dry, and incinerate in muffle furnace at  $600\text{--}650^\circ$  until all C is burned off. Cool, and add to residue from buret a quantity of 0.1 N  $\text{H}_2\text{SO}_4$  exactly equivalent to the  $\text{Na}_2\text{CO}_3$  originally added. Cover dish and place on steam bath 30 min. Filter, if necessary, into flask containing the first filtrate, washing paper thoroly with hot  $\text{H}_2\text{O}$  until free from acid. Cool soln and add 2 or 3 drops of methyl orange indicator (0.1 g per 100 ml  $\text{H}_2\text{O}$ ). If soln is alkaline, no further titration is necessary, and acidity is stated as "none." If soln is acid, titrate to distinct yellow color with the 0.1 N  $\text{Na}_2\text{CO}_3$  soln. Express result as percentage of  $\text{H}_2\text{SO}_4$ . With each set of determinations run blank thru entire procedure, using the standard solns. If blank is over 0.3 ml, repeat determinations.

9

EXTRACTION OF WATER-SOLUBLE MATERIAL<sup>8</sup>

Weigh 30 g of prepared leather, 1. (If fat content of sample, as determined by petroleum benzin extract, is more than 6%, extract 30 g charge with petroleum benzin, distilling at  $50\text{--}80^\circ$ , and allow petroleum benzin to evaporate spontaneously from charge before proceeding with extraction of water-soluble material.) Thoroly mix with charge sufficient  $\text{H}_2\text{O}$  to soak and cover leather. Transfer leather and extract to percolator that may be kept at  $50^\circ$ . Extract at  $50^\circ$  by percolating with  $\text{H}_2\text{O}$  at  $50^\circ$ , collecting 2 liters of percolate in 3 hours. Cool to room temp., dilute to exactly 2 liters, and mix thoroly.



To prevent fermentation add a few drops of toluene to prepared extract and reserve it for determination of glucose, soluble solids, and soluble non-tannins.

GLUCOSE<sup>9</sup>

10

## REAGENTS

(a) *Di-potassium phosphate*.—Use only  $K_2HPO_4$  that is practically free from primary and tertiary salts, has been dried in thin layers at 98–100° 16 hours, and kept in tightly stoppered bottles. A soln of the salt should have a pH value of ca 9.0 and give a barely perceptible pink color with phenolphthalein indicator.

(b) *Neutral lead acetate soln*.—See XXXIV, 19(d).

(c) *Soxhlet's modification of Fehling's soln*.—See XXXIV, 32.

(d) *Phenolphthalein soln*.—Dissolve 0.5 g of phenolphthalein in 100 ml of alcohol.

(e) *Tartaric acid*.—Grind pure tartaric acid to fine powder.

11

## PREPARATION OF SOLUTION

To 200 ml of prepared leather extract, 9, add by means of pipet 25 ml of the neutral Pb acetate soln. Shake frequently 5–10 min., filter at once thru a dry, folded filter, returning filtrate until it is clear. Keep containers and funnel covered during these operations. Add to filtrate 5.5 g of the dried  $K_2HPO_4$  (quantity of  $K_2HPO_4$  must be 4.5–6.5 g). Shake frequently 3–5 min., until all phosphate has dissolved. Filter thru dry, folded filter, returning first runnings until filtrate clears, and letting funnel drain well. Pipet 150 ml of the filtrate into 500 ml Erlenmeyer flask, and add by means of pipet 7.5 ml of HCl. Also add ca 25 mg of powdered stearic acid or 5–10 drops of kerosene to control frothing, and boil under reflux condenser exactly 2 hours. (If foaming occurs, turn off flame, and when foaming subsides relight immediately. No further trouble should be experienced. After hydrolysis the acid soln may stand at laboratory temp. overnight without risk of loss of sugar.) Cool to 10–15°, add 2 drops of the phenolphthalein indicator, carefully neutralize with NaOH (1+1) added from buret, and add 0.5 ml in excess. *Without delay* transfer soln to 200 ml volumetric flask, complete to volume with  $H_2O$ , and filter thru double filter, returning filtrate until it is clear. *During filtration keep filtrate just acid by addition from time to time of small quantities of pulverized pure tartaric acid. Immediately determine dextrose in soln.*

12

## DETERMINATION

Pipet 50 ml of the prepared soln into mixture of 25 ml of the Cu soln and 25 ml of the alkaline tartrate soln and proceed as directed under XXXIV, 38. Express results as percentage of glucose on leather basis, the 50 ml aliquot being equivalent to 0.5 g of leather.

13

## SOLUBLE SOLIDS

If  $H_2O$  extract, prepared as directed under 9, is clear, proceed as directed under XI, 2; if it is cloudy, proceed as directed under XI, 5.

14

## SOLUBLE NONTANNINS.—See XI, 8.

15

## SOLUBLE TANNIN

The percentage of soluble tannin is difference between 13 and 14.

16

## NITROGEN.—See II, 21.

17

## HIDE SUBSTANCE

Multiply percentage of N, 16, by factor 5.62, to convert to percentage of hide substance.

18

## COMBINED TANNIN

Deduct sum of percentages of moisture, 2 or 3; insoluble ash, 6; petroleum benzin extract, 7; soluble solids, 13; and hide substance, 17, from 100. The remainder is percentage of combined tannin.

## SELECTED REFERENCES

- <sup>1</sup> J. Am. Leather Chem. Assoc., 13, 232 (1918); 14, 321 (1919); 18, 154 (1923); 23, 412 (1928).  
<sup>2</sup> Ibid., 16, 547 (1921); 17, 262 (1922); 19, 568 (1924); 20, 334 (1925); 21, 435 (1926); 22, 265 (1927); J. Assoc. Official Agr. Chem., 10, 31, 143 (1927).  
<sup>3</sup> J. Am. Leather Chem. Assoc., 13, 7 (1918); 14, 243 (1919); 15, 130, 270 (1920).  
<sup>4</sup> Ibid., 16, 595 (1921); 17, 274, 592 (1922).  
<sup>5</sup> Ibid., 14, 140, 499, 507 (1919); 16, 458 (1921); 17, 292, 540 (1922); J. Soc. Leather Trades Chem., 4, 300 (1920).  
<sup>6</sup> Procter, Leather Industries Laboratory Book, 2nd ed., 1908, p. 369; J. Am. Leather Chem. Assoc., 14, 330 (1919).  
<sup>7</sup> J. Am. Leather Chem. Assoc., 17, 88 (1922); 18, 430 (1923); 23, 580 (1933); 29, 259 (1934).  
<sup>8</sup> Ibid., 11, 219 (1916); 13, 142 (1918); 14, 133, 488 (1919); 15, 581 (1920); 16, 124, 264, 491 (1921); 17, 220 (1922).  
<sup>9</sup> Ibid., 7, 645 (1912); 9, 421 (1914); 15, 411 (1920); 16, 480 (1921); 17, 284 (1922); 18, 262, 459 (1923); 19, 237, 339 (1924).

## XI. TANNING MATERIALS<sup>1</sup>—TENTATIVE

### EXTRACTS

1

#### PREPARATION OF SOLUTION

(a) *Solid and powdered extracts*.—Grind sample, if necessary, as rapidly as possible in porcelain mortar until all will pass 10-mesh sieve of Cu or brass; mix thoroly and bottle. Weigh rapidly a quantity of sample containing ca 4 g of tannin (not less than 3.75 nor more than 4.25 g).<sup>2</sup> Transfer to beaker containing 100 ml of H<sub>2</sub>O at 85°, place on steam bath, cover, and heat. Stir frequently until homogeneous soln or suspension is obtained. Wash into 1 liter flask with 800 ml of H<sub>2</sub>O at 85°. Allow to cool overnight at temp. not below 19°, bring to 20° by placing flask in H<sub>2</sub>O, the temp. of which is not below 19°, and dilute to 1 liter.

(b) *Liquid extracts*.—Let sample come to 20–30°, mix thoroly, and weigh rapidly charge yielding same quantity of tannin as is specified under (a). Dissolve by washing into liter flask with 900 ml of H<sub>2</sub>O at 85°. Allow to cool and dilute to 1 liter at 20°, as described under (a).

After preparation of soln proceed at once with analysis.

2

#### TOTAL SOLIDS

Thoroly mix prepared soln, 1, and pipet at once 100 ml into weighed flat-bottomed glass dish, 2½–3" in diameter. (1) Evaporate and dry 16 hours in combined evaporator and dryer<sup>3</sup> at 98–100°, or (2) evaporate on steam bath and dry 12 hours on bottom of water oven at 98–100°. Remove immediately to desiccator containing H<sub>2</sub>SO<sub>4</sub> (not more than 2 dishes in one desiccator) and weigh rapidly when cooled. Calculate percentage of total solids.

#### SOLUBLE SOLIDS

3

#### REAGENT

*Kaolin*.—Should be neutral to phenolphthalein and should not yield more than 1 mg of soluble solids per 100 ml of filtrate of 1% suspension in H<sub>2</sub>O after an hour's digestion at 20°.

4

#### PREPARATION OF FILTER

To ca 75 ml of prepared soln, 1, add 1 g of the kaolin. Stir, and pour immediately into single, 15 cm No. 590, S. & S. or No. 1F Swedish filter.<sup>4</sup> (These papers must be pleated by hand as they are not available in folded form.) Return filtrate to paper when ca 25 ml has run thru and repeat operation for hour, thus transferring all kaolin to paper. At end of an hour discard soln on filter by siphoning it off, disturbing the kaolin as little as possible. An ordinary wash bottle serves well for this purpose.

5

#### DETERMINATION

Bring ca 150 ml of original prepared soln, 1, to exactly 20°. Fill filter, prepared as directed under 4, with this soln and discard filtrate until it runs thru clear. Keep filter full, temp. of filtering soln at 20–25°, and the funnel and receiving vessel

covered. Pipet at once 100 ml of the clear filtrate into weighed dish, evaporate, and dry as directed under 2. Calculate percentage of soluble solids.

6

## INSOLUBLE SOLIDS

Percentage of insoluble solids = Total Solids (2) minus Soluble Solids (5).

## NONTANNINS

7

## REAGENT

*Hide powder.*<sup>5</sup>—Should be of woolly texture, well delimited, and 10 g of water-free powder should require 12–13 ml of 0.1 N NaOH to neutralize it.

Calculate quantity of air-dried hide powder that will be required for number of determinations to be made, on basis of 12.5 g of H<sub>2</sub>O-free powder for each determination. Increase this calculated amount by 10 g of air-dried hide powder to provide a sufficient quantity for determination of moisture in wet chromed hide powder and also for a working leeway.

Digest total quantity of air-dried hide powder with 10 times its weight of H<sub>2</sub>O until thoroly soaked. For each g of air-dried hide powder, so digested, add 1 ml of 3% chrome alum soln, K<sub>2</sub>SO<sub>4</sub>Cr<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·24H<sub>2</sub>O, and either agitate frequently several hours and let stand overnight, or agitate in some form of mechanical shaker an hour.<sup>6</sup> Transfer to strong linen filter and squeeze thoroly; using linen filter as a bag, leave hide powder in it and digest 15 min. with quantity of H<sub>2</sub>O equal to 15 times weight of air-dried hide powder used. Filter, and squeeze to ca 73% of H<sub>2</sub>O, using press if necessary. (Strong pressure is required to reduce H<sub>2</sub>O content below 70%.) Repeat digestion and filtration 3 times. The wet chromed hide powder, as finally prepared, should contain as nearly as possible 73% of H<sub>2</sub>O. Moisture content must be not less than 72% nor more than 74%.<sup>6</sup> Determine moisture in 20 g of squeezed hide powder as directed under 2.

8

## DETERMINATION

Place 46 g of prepared wet hide powder, 7, in shaker bottle of suitable capacity; add 200 ml of prepared tanning soln, 1, and shake immediately 10 min. in mechanical shaker. Squeeze at once thru linen; add 2 g of kaolin, 3, to filtrate that contains the nontannins; stir; and filter thru a single, *folded* 18.5 cm filter paper (No. 1F Swedish preferred), refiltering until filtrate is clear. Test filtrate with gelatin-salt soln (1% gelatin and 10% salt), and if precipitate forms, report fact. Pipet 100 ml of filtrate into weighed dish and evaporate as directed under 2. Correct weight of nontannin residue for dilution caused by H<sub>2</sub>O retained in wet hide powder. Calculate percentage of nontannins.

9

## TANNIN

Percentage of tannin = Soluble Solids (5) minus Nontannins (8).

SUGARS<sup>7</sup>

10

## PREPARATION OF SOLUTIONS

To 400 ml of prepared soln, 1, add 50 ml of Pb acetate soln, XXXIV, 19(d), shake well, and let stand 5–10 min. Filter thru folded filter (18.5 cm), returning filtrate until it is clear. Let the filter drain ca 30 min. after all soln has been poured. Remove excess Pb from filtrate with dried K<sub>2</sub>HPO<sub>4</sub>, X, 10(a), using phosphate in proportion of 5 g to 200 ml of filtrate. (Measure filtrate in graduated cylinder, usually 360–380

ml will be obtained, requiring 9–9.5 g of  $K_2HPO_4$ . Weigh phosphate to within 0.1 g.) After adding phosphate, shake well 4 or 5 min. and filter thru folded filter (18.5 cm).

## 11

## DETERMINATION

(a) *Reducing sugars*.—Place in flask 100 ml of clarified delead ed soln, 10, add 33.3 ml of  $H_2O$ , and if reduction is not made at once, 8–10 drops of toluene; shake well and stopper with plug of cotton. Keep in cool place and make reduction within 24 hours. When ready for reduction, filter if toluene has been added. Determine reducing sugars in duplicate 50 ml aliquots, as directed under XXXIV, 38. After correcting weight of  $Cu_2O$  for blank of the Fehling soln, find equivalent mg of dextrose from XLIII, 9. To express as percentage of dextrose, multiply mg of dextrose by 3 and divide result by g of sample per liter of prepared soln, 1.

(b) *Total sugars*.—Place in 500 ml Erlenmeyer flask 150 ml of clarified, delead ed soln, 10, add 7.5 ml of HCl, and boil under reflux condenser exactly 1 hour. (If foaming occurs, add 5–10 drops of kerosene.) After boiling an hour, remove flask, stopper loosely when moderately cool, and let stand until ready for reduction, usually overnight. Cool soln in ice  $H_2O$  20–30 min., add 2 drops of phenolphthalein soln X, 10(d), carefully neutralize with NaOH (1+1), and add HCl dropwise until color of indicator is just discharged. After bringing soln to room temp., transfer to 200 ml flask, make to mark, mix, and filter until clear. Reduce the Fehling soln with duplicate 50 ml aliquots and calculate results as directed under (a).

(c) *Non-reducing sugars*.—Percentage of non-reducing sugars is difference between Reducing Sugars, 11(a), and Total Sugars, 11(b).

DETECTION OF SULFITE-CELLULOSE<sup>8</sup>

## 12

## REAGENT

*Sulfite-cellulose soln*.—Dissolve 0.5 g of the total solids derived from sulfite-cellulose in 1 liter of  $H_2O$  and add sufficient tanning material, free from sulfite-cellulose, to give concentration of 3.75–4.25 g of tannin per liter.

## 13

## DETERMINATION

Place 5 ml of prepared tanning soln, 1, in test tube. Add 0.5 ml of aniline and shake well; add 2 ml of HCl and mix again. Compare precipitate formed with that produced when sulfite-cellulose soln is similarly treated. In predetermined absence of the synthetic tanning materials known as syntans, sulfite-cellulose is considered to be present if volume of precipitate approximately equals or exceeds that of comparison soln.

## LIQUORS

## 14

## PREPARATION OF SOLUTION

Dilute the liquor with  $H_2O$  at 20–30° to contain ca 0.7 g of solids in 100 ml of soln. If liquor does not give proper soln with  $H_2O$  at 20–30°, dilute with  $H_2O$  at 80° and cool to 20°, as directed under 1(a).

## 15

## TOTAL SOLIDS.—See 2.

## 16

## SOLUBLE SOLIDS.—See 5.

## 17

## NONTANNINS

Proceed as directed under 8, using quantity of wet chromed hide powder that will give ratio between tannin and hide powder shown in following table:<sup>9</sup>

TANNIN RANGE PER 100 ML	DRY HIDE POWDER PER 200 ML
<i>gram</i>	<i>gram</i>
0.35-0.45	9.0-11.0
0.25-0.35	6.5- 9.0
0.15-0.25	4.0- 6.5
0.00-0.15	0.0- 4.0

## TOTAL ACIDITY

18

## REAGENTS

(a) *Hematin indicator*.—Digest 0.5 g of hematin in 100 ml of cold neutral alcohol.

(b) *Gelatin soln*.—Soak 10 g of gelatin in H<sub>2</sub>O at room temp. 1-2 hours and warm slightly, not exceeding 50°, to complete soln; add 25 ml of alcohol, and dilute. If gelatin soln is acid or alkaline, neutralize with 0.1 N NaOH or 0.1 N acetic acid, respectively, using hematin indicator, and dilute to 1 liter.

(c) *Kaolin*.—Digest with HCl (1+9), wash until it complies with tests given under 3, dry, and preserve in a tightly stoppered bottle.

19

DETERMINATION<sup>10</sup>

Add 50 ml of the gelatin soln to 25 ml of the tanning liquor in a stoppered cylinder; dilute with H<sub>2</sub>O to 250 ml, add 15 g of the kaolin, and shake vigorously. Allow to settle at least 15 min., remove 30 ml of supernatant liquid, dilute with 50 ml of H<sub>2</sub>O, and titrate with 0.1 N NaOH, using the hematin indicator. 1 ml of 0.1 N NaOH = 0.2% of acid, calculated as acetic, in liquor.

## RAW AND SPENT MATERIALS

(Under raw materials are included woods, barks, leaves, etc.)

20

## MOISTURE IN SAMPLE AS RECEIVED

Cut or break up large pieces and mix sample rapidly to avoid change in moisture content. Dry as directed under 2, a suitable weighed quantity, dependent upon physical condition and moisture content of sample.

21

## PREPARATION OF SAMPLE

Dry remainder of sample at temp. not above 60°, and grind to pass thru 20-mesh sieve.

22

## MOISTURE IN PREPARED SAMPLE

Dry 10 g of prepared sample, 21, as directed under 2, and calculate all results to "as-received," "air-dried," or "moisture-free" basis, as desired.

23

## EXTRACTION

(a) *Woods, barks, and spent materials*.—Weigh quantity of sample that will give an extract containing as nearly as possible 4 g of tannin per liter. Transfer to beaker and wet thoroly with hot H<sub>2</sub>O. Place perforated porcelain plate in tin-lined Cu extractor of general form shown in Fig. 15; on plate place layer of cotton and wet thoroly with H<sub>2</sub>O. Connect extractor with 1000 ml Erlenmeyer flask (G) containing 800 ml of H<sub>2</sub>O, close stopcock E, connect D by delivery tube to 1000 ml

graduated flask and close D. Wash material into extractor with minimum amount of hot  $H_2O$ . Open D and return percolate thru extractor 2 or 3 times, or until it is practically clear. Place layer of cotton on top of the material. Connect metal cap B and condenser A so that condensate will drop onto layer of cotton. Boil water in G and collect 500 ml of percolate in the graduated flask in 2 hours. Close D, open E, and continue boiling 14 hours, applying heat so that ca 330 ml of  $H_2O$  will be condensed per hour. Transfer extract in G to graduated 1 liter flask, heat to  $80^\circ$ , cool as directed under 1(a) and dilute to mark.

(b) *Materials other than woods, barks, and spent materials.*—Weigh quantity of sample sufficient to give 2 liters of extract containing 4 g of tannin per liter. Place in extractor described under (a), digest 1 hour with  $H_2O$  at room temp., and start the extraction. Keep stopcock E closed and collect entirely thru the side tube D 2 liters of percolate in ca 7 hours. Heat percolate to  $80^\circ$ , cool as directed under 1(a), and dilute to mark.

#### 24 ANALYSIS OF EXTRACT

Proceed as directed under 2-9, inclusive. With solns more dilute than specified (often the case with spent materials) reduce quantity of hide powder used in determination of nontannins in accordance with concentration of soln and schedule given under 17.

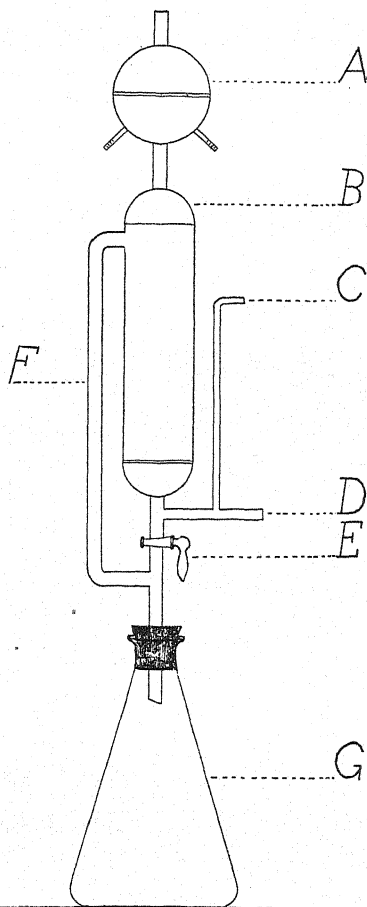


FIG. 15.—APPARATUS FOR EXTRACTING TANNING MATERIALS

#### SELECTED REFERENCES

<sup>1</sup> The proceedings of the A.O.A.C. that deal with the early development of the methods of analysis of tanning materials will be found in Bureau of Chemistry Bulletins Nos. 43, 47, 49, 51, 56, 62, 67, 73, 81, and 90. These have been assembled in J. Am. Leather Chem. Assoc., 15, 1a-127 (1920).

<sup>2</sup> J. Am. Leather Chem. Assoc., 7, 288, 296 (1912).

<sup>3</sup> Ibid., 1, 32 (1906); 9, 442 (1914).

<sup>4</sup> Ibid., 10, 282 (1915).

<sup>5</sup> The official hide powder of the American Leather Chemists Association is prepared only by the Standard Manufacturing Company, Ridgeway, Pa.

<sup>6</sup> J. Am. Leather Chem. Assoc., 7, 292 (1912).

<sup>7</sup> Ibid., 23, 91 (1928).

<sup>8</sup> Ibid., 9, 36, 130 (1914).

<sup>9</sup> Ibid., 5, 5 (1910).

<sup>10</sup> Ibid., 3, 85 (1908); 4, 194 (1909).

## XII. PLANTS

1

### DIRECTIONS FOR SAMPLING—OFFICIAL

When more than one plant is sampled, include in sample a sufficient number of plants to insure that it represents adequately average composition of entire lot of plants sampled. This number cannot be stated definitely; it will depend upon the variability in composition of the plants. Details of procedure must be determined by purpose for which sample is taken.

2

### PREPARATION OF SAMPLE—OFFICIAL

(a) *For mineral constituents.*—Thoroughly remove all foreign matter from material, especially adhering soil or sand, avoiding excessive washing to prevent leaching. Air dry as rapidly as possible to prevent decomposition or loss in weight by respiration, grind, and preserve in tightly stoppered bottles. If results are to be expressed on fresh weight basis, record weights of sample before and after air drying. When determinations of Cu, Mn, Zn, Fe, Al, etc., are to be made, take precautions to prevent contamination of sample by dust during air drying and from grinding and sieving machinery.

(b) *For carbohydrates.*—Thoroughly remove all foreign matter and rapidly grind or chop material into fine pieces. Add weighed sample to sufficient hot redistilled alcohol to which sufficient precipitated  $\text{CaCO}_3$  has been added to neutralize acidity, using sufficient alcohol so that final concentration, allowing for  $\text{H}_2\text{O}$  content of sample, will be ca 80%. Heat close to b.p. on steam or water bath 30 min., stirring frequently. Samples may be stored until needed for analysis.

3

### MOISTURE—TENTATIVE.—See XXVII, 2, 6, or 7.

4

### ASH—TENTATIVE.—See XXXIV, 9 or 10; XXVII, 8.

5

### SAND AND SILICA—OFFICIAL

Ignite 10–50 g of substance in flat-bottomed Pt dish in muffle, at temp. not exceeding dull redness, until residue is white or nearly so. Moisten with 5–10 ml of HCl, boil ca 2 min., evaporate to dryness, and heat on steam bath 3 hours to render the  $\text{SiO}_2$  insoluble. Moisten residue with 5 ml of HCl, boil 2 min., add ca 50 ml of  $\text{H}_2\text{O}$ , heat on water bath a few minutes, filter thru hardened filter, and wash thoroughly. To this filtrate add filtrate and washings from alkali-soluble  $\text{SiO}_2$  determination (below) and dilute to 200 ml. Designate as soln A.

(a) *Sand.*—Wash the residue from filter into Pt dish and boil ca 5 min. with ca 20 ml of a saturated soln of  $\text{Na}_2\text{CO}_3$ , add a few drops of 10% NaOH soln, allow mixture to settle, and decant thru ignited and weighed Gooch crucible. Boil residue in dish with another 20 ml portion of the  $\text{Na}_2\text{CO}_3$  soln and decant as before. Repeat process. Transfer residue to Gooch crucible and wash thoroughly, first with hot  $\text{H}_2\text{O}$ , then with a little HCl (1+4), and finally with hot  $\text{H}_2\text{O}$  until free from chlorides. Dry filter and contents, ignite, and weigh as sand. Confirm by microscopical examination.

(b) *Alkali-soluble  $\text{SiO}_2$ .*—Combine alkaline filtrate and washings, acidify with HCl, evaporate to dryness, add 5 ml of HCl, again evaporate, and dehydrate by heating to 110–120° 2 hours. Moisten residue with 5–10 ml of HCl, boil ca 2 min., add ca 50 ml of  $\text{H}_2\text{O}$ , heat on water bath 10–15 min., filter thru ashless filter or



ignited and weighed Gooch crucible, wash with hot  $\text{H}_2\text{O}$ , ignite, and weigh as  $\text{SiO}_2$ . Add filtrate to soln A.

6

IRON AND ALUMINUM<sup>2</sup>—OFFICIAL

Take aliquot of soln A, 5, containing ca 40 mg of Fe- and  $\text{AlPO}_4$ . Oxidize the Fe. If soln does not already contain an excess of phosphate, add to aliquot containing ca 40 mg of Fe- and  $\text{AlPO}_4$ , 0.5 g of  $(\text{NH}_4)_2\text{HPO}_4$ , stir until dissolved, and make up to 50 ml with  $\text{H}_2\text{O}$ . Add a few drops of thymol blue and then add  $\text{NH}_4\text{OH}$  until soln just turns yellow. Run in 0.5 ml of  $\text{HCl}$ , follow with 25 ml of 25%  $\text{NH}_4$  acetate, and stir. Let stand at room temp. until precipitate settles (ca 1 hour). Filter, and wash 10 times with hot 5%  $\text{NH}_4\text{NO}_3$  soln. Ignite, and weigh as Fe- and  $\text{AlPO}_4$ .

Fuse ignited precipitate in Pt crucible with ca 4 g of a mixture of equal parts of  $\text{Na}_2\text{CO}_3$  and  $\text{K}_2\text{CO}_3$ . When fusion is complete, allow crucible to cool, add 5 ml of  $\text{H}_2\text{SO}_4$ , and heat until copious fumes of  $\text{SO}_3$  are given off. Cool, transfer to flask, add  $\text{H}_2\text{O}$ , and digest until soln is clear. Reduce Fe with Zn, cool, and titrate with 0.1 N  $\text{KMnO}_4$  soln. Report as percentage of  $\text{Fe}_2\text{O}_3$ . Calculate the oxide to phosphate and subtract from total Fe- and  $\text{AlPO}_4$ . This gives the  $\text{AlPO}_4$ . Report as  $\text{Al}_2\text{O}_3$ .

## METHOD FOR IRON ONLY

7

*Colorimetric Method<sup>3</sup>—Official*

To an aliquot of soln containing ca 0.2 mg of Fe, add  $\text{H}_2\text{O}$  to make ca 40 ml, 5 ml of  $\text{HCl}$ , and 0.3 ml of  $\text{HNO}_3$ , and boil ca 30 min. Transfer mixture to 50 ml volumetric flask, add  $\text{H}_2\text{O}$  to make ca 35 ml, cool, add 10 ml of 20%  $\text{KSCN}$  soln, fill to mark, and compare intensity of color with that of a standard containing somewhere near same amount of Fe as sample.

8

*Titrimetric Method<sup>4</sup>—Tentative*

Prepare sample as directed under 7. Take a convenient aliquot of the soln, oxidize the Fe by adding dropwise a soln of  $\text{KMnO}_4$  (1+100) until a very, very faint permanganate color persists. Add 5 ml of 10%  $\text{NH}_4\text{CNS}$  and titrate with ca 0.02 N  $\text{TiCl}_3$  to disappearance of red color. (The  $\text{TiCl}_3$  should be prepared, standardized with a known iron soln, kept in dark in well-filled container, and standardized against the Fe soln each time it is used or every few hours when making a large number of determinations. This is easily and quickly done if a standard iron soln is kept on hand.)

9

MICRO METHOD FOR ALUMINUM ONLY<sup>4</sup>—TENTATIVE

Take an aliquot of soln A, 5, containing ca 0.05 mg of Al. Oxidize the Fe by boiling with a few drops of  $\text{HNO}_3$  and transfer to conical centrifuge tube of ca 25 ml capacity with marks at 15, 20, and 25 ml. If quantity of Fe is very small, add  $\text{Fe}_2\text{Cl}_6$  soln equivalent to ca 1 mg of iron; or if quantity of phosphate present is small, add ca 0.1 g of  $(\text{NH}_4)_2\text{HPO}_4$  to insure complete precipitation of the Fe and Al. Dilute contents to ca 15 ml with  $\text{H}_2\text{O}$ , neutralize with  $\text{NH}_4\text{OH}$ , using a drop of dilute methyl red as indicator, and add 1 ml of a saturated soln of  $\text{NH}_4$  acetate. Place tube in water bath until precipitate begins to settle (ca 10 min.), centrifuge, decant, and discard supernatant liquid. Dissolve precipitate in 1 ml of ca 6 N  $\text{HCl}$  with warming when necessary and dilute to 15 ml. Cool, add 1.25 ml of glacial acetic acid and 5 ml of 6 N  $\text{NaOH}$  (special Al-free), wash down sides, and fill to 25 ml mark. Let stand ca 1 hour and centrifuge. The precipitate contains the Fe and the soln the Al.

Transfer to 50 ml volumetric flask as large an aliquot as can be drawn off. Add  $\text{H}_2\text{O}$  to make ca 20 ml, a small piece of litmus paper, and finally  $\text{HCl}$  (1+9) until litmus paper just turns red. Determine Al as follows: add 5 ml of 5  $N$   $\text{NH}_4$  acetate, 5 ml of 1.5  $N$   $\text{HCl}$ , and 2 ml of 0.1 % of the dye Aluminon (ammonium salt of aurintricarboxylic acid) and place in water bath at ca  $80^\circ$  for 10 min. Add 5 ml of 5  $N$   $\text{NH}_4\text{Cl}$ , cool to room temp., add 5 ml of 3.2  $N$   $(\text{NH}_4)_2\text{CO}_3$  while shaking gently, fill to mark with  $\text{H}_2\text{O}$ , and mix. (At this point reaction should be 7.1–7.3 and red color of blank should disappear in ca 15 min. The exact concentration of reagents is not important, but final pH is, and amount of  $(\text{NH}_4)_2\text{CO}_3$  necessary to bring soln to above pH should be determined by neutralizing similar solns without adding the dye.) Simultaneously with above procedure run a standard (or standards if necessary) containing given quantity of Al. After allowing mixture to stand 20 min. for excess dye to decolorize, compare color intensities and read amount of Al from a curve plotted as described in following paragraph.

If only a small number of determinations are to be made, prepare 4 standards containing 0.01, 0.03, 0.05 and 0.07 mg of Al, respectively, and run these with samples. Compare all these solns with standard containing 0.03 mg of Al and calculate results to colorimeter reading of 30 for this standard. Arbitrarily give 0.005 mg of Al a reading of 100 and with this and the 4 readings on standards plot a curve. Read quantity of Al in each sample from this curve. If the determinations are to extend over a period of time, it is advisable to make them on several series of standards and plot a curve from the average of these results. It is then necessary to run only 1 standard each time determinations are to be made, and results can be read from curve. Blanks must be run on both the Fe and Al determinations as nearly all reagents contain traces of these elements.

#### CALCIUM—OFFICIAL

10

##### *Macro Method<sup>b</sup>*

(Applicable to material containing less than 0.05% of  $\text{MnO}$ .)

Transfer aliquot of soln A, 5, to 200 ml beaker, add  $\text{H}_2\text{O}$  if necessary to make to 50 ml, heat to boiling, and add 10 ml of saturated soln of  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  and a drop of methyl red. Almost neutralize with  $\text{NH}_4\text{OH}$  and boil until precipitate is coarsely granular. Cool, add  $\text{NH}_4\text{OH}$  (1+4) until color is faint pink (pH 5.0) and allow to stand at least 4 hours. Filter, and wash with  $\text{H}_2\text{O}$  at room temp. until filtrate is free from oxalates. Break point of filter with a Pt wire and wash precipitate into beaker in which the Ca was precipitated with hot  $\text{H}_2\text{SO}_4$  (1+4) and hot  $\text{H}_2\text{O}$ . Add ca 10 ml of  $\text{H}_2\text{SO}_4$  (1+4), heat to ca  $90^\circ$ , add ca 50 ml of hot  $\text{H}_2\text{O}$ , and titrate with 0.05  $N$   $\text{KMnO}_4$ . Finally add filter paper to soln and complete titration.

11

##### *Micro Method<sup>b</sup>*

Ignite 2 g of substance in small crucible in muffle at dull red heat. Dissolve ash in  $\text{HCl}$  (1+4) and transfer to 100 ml beaker. Add 5 ml of  $\text{HCl}$  and evaporate to dryness on steam bath to dehydrate the  $\text{SiO}_2$ . Moisten residue with 5 ml of  $\text{HCl}$ , add ca 50 ml of  $\text{H}_2\text{O}$ , heat a few minutes on steam bath, transfer to 100 ml volumetric flask, cool quickly to room temp., make to volume, shake, and filter, discarding first portion of filtrate. Pipet 15 ml aliquot into conical-tipped centrifuge tube containing 2 ml of saturated  $\text{NH}_4$  oxalate soln and 2 drops of 0.05% methyl red. Add 2 ml of acetic acid (1+4), rotating tube to mix contents thoroly. Add, while intermittently rotating tube,  $\text{NH}_4\text{OH}$  (1+4) until soln is faintly alkaline, after which add a few drops of the acetic acid with dropper until color is adjusted to faint pink

(pH 5.0). (It is important at this point to rotate tube so that last bit of liquid in conical tip is color required.) Allow mixture to stand at least 4 hours and whirl tube in centrifuge 15 min. (Precipitate should then be in a firm lump in tip of tube.) Remove supernatant liquid by means of the suction device (Fig. 16), taking care not to disturb precipitate. Wash precipitate by adding 2 ml of  $\text{NH}_4\text{OH}$  (1+49), rotating tube to break up precipitate. (It may be necessary to jar tube sharply.) Return tube to centrifuge 10 min., and again remove supernatant liquid and wash with reagent as before. Repeat this operation until precipitate has been washed 3

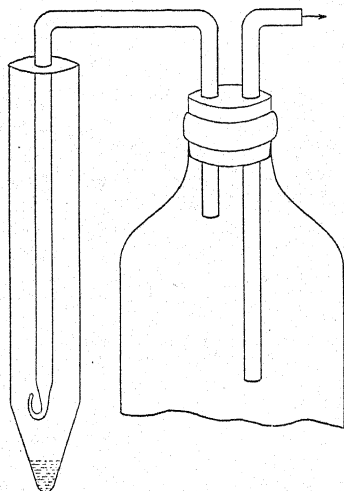


FIG. 16.—SUCTION DEVICE USED IN MICRO METHOD FOR DETERMINATION OF CALCIUM

times. When supernatant liquid has been removed after final centrifuging add 2 ml of  $\text{H}_2\text{SO}_4$  (1+4) to tube, break up precipitate as before, heat on steam bath to  $80-90^\circ$ , and titrate in tube with 0.02 N  $\text{KMnO}_4$ , rotating liquid during titration to attain a proper end point. If tube cools below  $60^\circ$  during addition of the  $\text{KMnO}_4$ , reheat it in steam bath a few minutes and complete titration. Run blank on identical amount of  $\text{H}_2\text{SO}_4$  in a similar tube heated to same temp. to determine quantity of 0.02 N  $\text{KMnO}_4$  necessary to give color of end point. Subtract this value from buret reading. 1 ml of 0.02 N  $\text{KMnO}_4 = 0.0004$  g of Ca. Report as percentage of Ca.

## 12

### MAGNESIUM<sup>7</sup>—OFFICIAL

To combined filtrate and washings from Ca determination, 10, add 30 ml of  $\text{HNO}_3$  and evaporate to dryness to decompose the  $\text{NH}_4$  salts. Take up with 5 ml of  $\text{HCl}$  and make to ca 100 ml with  $\text{H}_2\text{O}$ . Add 5 ml of 10% Na citrate soln and 10 ml of 10%  $(\text{NH}_4)_2\text{HPO}_4$  soln, or enough to precipitate all the Mg. Add

$\text{NH}_4\text{OH}$  (1+4) with constant stirring (using policeman) until soln is faintly alkaline and precipitate forms; then add 25 ml of  $\text{NH}_4\text{OH}$ , stir vigorously until precipitate is granular, and allow to stand in cool place overnight. Filter and wash free from chlorides with cold  $\text{NH}_4\text{OH}$  (1+10). Ignite, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Report as percentage of  $\text{MgO}$ .

## 13

### MANGANESE<sup>3</sup>—OFFICIAL

To aliquot of soln A, 5, representing 0.2–0.5 g of ash, add 15 ml of  $\text{H}_2\text{SO}_4$  and evaporate to ca 30 ml. Add 5–10 ml of  $\text{HNO}_3$  and continue evaporation. (It is neither necessary nor advisable to evaporate until dense fumes appear, since the  $\text{Fe}_2(\text{SO}_4)_3$  then dissolves with difficulty.  $\text{HNO}_3$  may be present, but not  $\text{HCl}$ .) Add  $\text{H}_2\text{O}$ , a little at a time, heat until the Fe salts have dissolved, and dilute to ca 150 ml. Add 0.3 g of  $\text{KIO}_4$  in small portions, boil a few minutes or until the color of the  $\text{KMnO}_4$  shows no further increase in intensity, and allow to cool.

Prepare standard as follows: To volume of  $\text{H}_2\text{O}$  equal to sample add 15 ml of  $\text{H}_2\text{SO}_4$  and sufficient pure  $\text{Fe}(\text{NO}_3)_3$ , free from Mn, to equal ca the quantity of Fe in sample. Add measured quantity of 0.1 N  $\text{KMnO}_4$  soln until color is slightly darker than sample, add 0.3 g of  $\text{KIO}_4$  and boil a few minutes. When cool, transfer sample and standard to 250 ml flasks and dilute to mark with  $\text{H}_2\text{O}$ . If color is weak, it may

be necessary to dilute to less than 250 ml. Compare colors in any standard colorimeter. Report results as percentage of  $Mn_2O_4$ .

14

## SODIUM AND POTASSIUM—OFFICIAL

Moisten 1–10 g of sample with  $H_2SO_4$  (1 + 10), dry in oven, and ignite in muffle at a low red heat to destroy organic matter. Heat residue on steam bath with 2–5 ml of HCl and ca 50 ml of  $H_2O$ . Transfer to beaker and add  $NH_4OH$  dropwise until precipitate formed requires several seconds to dissolve, thus leaving soln but faintly acid. Heat nearly to boiling, and add  $NH_4OH$  to precipitate all the Fe, Al, etc. Boil in covered beaker ca 1 min.; remove, and if no  $NH_3$  is detected by smelling, continue addition, dropwise, until it can be detected. Do not allow precipitate to settle, but stir and pour on filter. Wash immediately with hot  $H_2O$ , using, to effect rapid filtration, a fine jet directed around edge of precipitate to cut it free from the paper. Wash precipitate several times, return to original beaker, dissolve with a few drops of HCl, and warm. Reprecipitate the Fe, Al, and  $P_2O_5$  with  $NH_4OH$  as directed above; filter and wash until free from chlorides. Evaporate combined filtrates and washings to dryness, heat below redness until  $NH_4$  salts are expelled, and dissolve in hot  $H_2O$ . Add 5 ml of a saturated soln of  $Ba(OH)_2$ , heat to boiling, allow to settle a few minutes, and determine whether or not precipitation is complete by addition of more of the  $Ba(OH)_2$  soln to a little of the clear liquid. When no further precipitate is produced, filter and wash thoroly with hot  $H_2O$ . Heat filtrate to boiling and add  $NH_4OH$  (1 + 4) and a 10%  $(NH_4)_2CO_3$  soln to complete precipitation of the Ba, Ca, etc. Let stand short time on water bath, filter, and wash precipitate thoroly with hot  $H_2O$ . Evaporate filtrate and washings to dryness, expel  $NH_4$  salts by heating below redness, treat with a little hot  $H_2O$ , and add a few drops of the dilute  $NH_4OH$ , 1 or 2 drops of the  $(NH_4)_2CO_3$  soln, and a few drops of a saturated soln of  $NH_4$  oxalate. Let stand a few minutes on water bath and set aside a few hours. Filter, evaporate to complete dryness on water bath, and heat at temp. not exceeding dull redness until all  $NH_4$  salts are expelled and residue is nearly or quite white. Dissolve in minimum quantity of  $H_2O$ , filter into weighed Pt dish, add a few drops of HCl, evaporate to dryness on water bath, heat at temp. not exceeding dull redness, cool in desiccator, and weigh as KCl plus NaCl. Repeat the heating until constant weight is obtained.

15

*Platinic Chloride Method for Potassium—Official*

Dissolve residue of mixed chlorides, 14, with a few ml of  $H_2O$ , acidify with a few drops of HCl, and add excess of  $PtCl_4$  soln, II, 40(b). Evaporate on water bath to thick paste; treat residue repeatedly with 80% alcohol, decanting thru a weighed Gooch crucible or other form of filter, transfer precipitate to filter and wash thoroly with the 80% alcohol. Dry 30 min. at  $100^\circ$  and weigh.  $K_2PtCl_6 \times 0.16084 = K$ . If it is desired to determine the Na, calculate the K to KCl and subtract this from the KCl + NaCl found in preceding paragraph.

16

*Perchloric Acid Method for Sodium and Potassium<sup>3</sup>—Tentative*

Prepare material as directed in 14 until heavy metals have been removed and the two elements are in the form of chlorides. (Sulfates must be absent.) Add 3–5 ml of 60%  $HClO_4$ . Evaporate to dryness, dissolve in hot  $H_2O$ , and again evaporate to dryness. Heat to  $350^\circ$ , cool, and weigh if it is desired to obtain combined perchlorates. Add 10–20 ml of a mixture of anhydrous ethyl acetate and C.P. normal butyl alcohol in equal proportions by volume. Digest near b.p. several minutes. Decant into Gooch crucible. Wash once or twice by decantation with a few ml of the acetate-alcohol mixture. Dissolve in minimum quantity of  $H_2O$ , evaporate to dryness, and

extract as before. Filter, and wash several times with 1 ml of the acetate-alcohol mixture. Dry in oven at 110° several minutes and heat at 350° 15 min. Cool, and weigh.  $\text{KClO}_4 \times 0.28218 = \text{K}$ . Calculate Na by difference.

17

*Rapid Method for Potassium Only—Official*

Proceed as directed under 14 to "and if no  $\text{NH}_3$  is detected . . . until it can be detected." Add a few ml of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$ , let stand a few hours, filter, and wash with hot  $\text{H}_2\text{O}$  until free from chlorides. Concentrate to small volume, transfer to Pt evaporating dish, evaporate to drive off excess  $\text{NH}_3$ , add 0.5 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate, ignite by whirling dish over a free flame, and proceed as directed under 15.

*Magnesium Uranyl Acetate Method for Sodium Only<sup>a</sup>—Tentative*

18

## REAGENT

*Magnesium uranyl acetate soln.*

(a) *Crystallized uranyl acetate.*—To 85 g add 60 g of glacial acetic acid and  $\text{H}_2\text{O}$  to make 1000 ml.

(b) *Crystallized magnesium acetate.*—To 500 g add 60 g of glacial acetic acid and  $\text{H}_2\text{O}$  to make 1000 ml.

Heat (a) and (b) separately to ca 70° until all salts are dissolved. Mix two solns at this temp. and allow to cool to ca 30°. Place vessel containing mixed reagent in  $\text{H}_2\text{O}$  at 20°, and hold at this temp. 1–2 hours, or until the slight excess of salts is crystallized out. Filter reagent thru dry filter into dry bottle.

19

## DETERMINATION

Moisten 1–10 g of sample with  $\text{H}_2\text{SO}_4$  (1+10), dry in oven, and ignite in muffle at low red heat to destroy organic matter. Heat residue on steam bath with 2–5 ml of  $\text{HCl}$ , add ca 40 ml of  $\text{H}_2\text{O}$ , and heat to boiling. Add sufficient  $\text{CaCl}_2$  soln to precipitate all phosphates. Precipitate phosphates by making slightly alkaline with  $\text{NH}_4\text{OH}$ . Filter, and evaporate to 5 ml or less if no salts separate. Cool. Add 100 ml of the Mg uranyl acetate soln, place mixture in water bath at 20°, and either stir vigorously 45 min. or let stand 24 hours. Filter with suction and wash with alcohol saturated with the Na-Mg-uranyl acetate. Dry at 105–110° 30 min., cool, and weigh. Weight of Na-Mg-uranyl acetate  $\times 0.0153 = \text{Na}$ .

COPPER<sup>10</sup>—OFFICIAL, FIRST ACTION

20

## REAGENTS

(a) *Potassium ethyl xanthate.*—0.1% water soln prepared fresh each time it is used.

(b) *Standard copper sulfate.*—Dissolve 0.3928 g of pure  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , dilute in volumetric flask to 1000 ml, and mix. 1 ml = 0.0001 g of Cu.

(c) *Filter-paper pulp.*—Moisten and tear a good grade of sheet filter paper into bits and place in porcelain dish. Add, while stirring with glass rod, enough cold  $\text{HCl}$  to disintegrate paper and reduce mass to mushy consistency. Transfer pulp to large Büchner funnel and wash free of acid, using suction. Transfer washed pulp to clean glass bottle and add  $\text{H}_2\text{O}$  to make a thick pulp suitable for making a pad in a Caldwell crucible. (Precipitates of Cu and Zn obtained in these methods can be readily filtered and washed upon pads made with this filter-paper pulp by use of suction pump.)

21

## DETERMINATION

Ash 100–500 g of finely divided air-dried plant material in  $\text{SiO}_2$  dishes with a small flame, but do not allow plant material to burn with a blaze. After volatile matter has been dispelled, complete ashing in a muffle furnace maintained at temp. of a faint red glow. Hasten ashing process by removing dishes from muffle at intervals and breaking up lumps with a Pt stirring rod. After C has been oxidized as completely as possible, cool dish, moisten ash with  $\text{H}_2\text{O}$ , wash into a 250 ml beaker, and cover with watch-glass. Decompose ash with  $\text{HCl}$  (1+1) introduced thru lip of beaker beneath watch-glass by means of a pipet. After effervescence has ceased, rinse watch-glass into beaker, filter insoluble residue on a Büchner funnel, and wash free of chlorides. If insoluble residue contains undecomposed particles of C, transfer it to a  $\text{SiO}_2$  dish and reignite in muffle furnace until all particles of C are decomposed and a light colored ash remains. Redigest ash on hot water bath with 15 ml of  $\text{HCl}$  (1+1), filter, and wash free of chlorides. Combine filtrates in a clean porcelain dish, evaporate to dryness, and bake at  $110^\circ$  until  $\text{HCl}$  is expelled. Moisten dry residue with 10 ml of  $\text{HCl}$  (1+1) and digest, with stirring, 10 min. Dilute with hot  $\text{H}_2\text{O}$ , filter out the  $\text{SiO}_2$ , wash free of chlorides, combine with insoluble residue, ignite, and weigh. Make filtrate to ca 250 ml, heat nearly to boiling point, and pass a slow stream of  $\text{H}_2\text{S}$  thru soln 15 min. Rinse the  $\text{H}_2\text{S}$  delivery tube into flask, tightly stopper, and set aside until precipitate settles and supernatant soln is clear. Filter the  $\text{CuS}$  on a pad of the paper pulp and wash with  $\text{HCl}$  (0.25 N) saturated with  $\text{H}_2\text{S}$ . Ignite in a porcelain crucible and dissolve the  $\text{CuO}$  in a few drops of  $\text{HNO}_3$  (1+9) and one drop of  $\text{HCl}$  (1+9). Filter soln and wash filter paper clean. Evaporate soln to dryness in a porcelain dish 3 times with the addition of a few drops of  $\text{HNO}_3$  (1+9) and take up with a very small drop of the  $\text{HNO}_3$  delivered from a stirring rod having a sharp point. Make to 50 ml, and transfer an aliquot of 5 ml to a Nessler tube containing 10 ml of the K ethyl xanthate. Mix solns and dilute to 25 ml. Transfer 10 ml of the K ethyl xanthate to a second Nessler tube, dilute to ca 15 ml, and add the standard  $\text{Cu}$  soln, dropwise with thoro mixing with a glass stirring rod, until color in standard tube apparently matches color in tube containing sample. Make final adjustment of volume and color of the standard in the tube containing the standard. Record number of ml of standard  $\text{Cu}$  soln required and report as percentage of  $\text{Cu}$ .

To filtrate from the  $\text{CuS}$  add 5 ml of  $\text{HNO}_3$  (1+1) and boil 10 min. to oxidize remaining metals. Cool soln and make to convenient volume (250 ml). From this stock soln take suitable aliquots for determination of  $\text{Zn}$  or other elements contained in ash of sample.

ZINC<sup>10</sup>—OFFICIAL, FIRST ACTION

22

## REAGENTS

(a) *Potassium ferrocyanide soln.*—Dissolve 2 g in  $\text{H}_2\text{O}$  and dilute to 100 ml. Should be freshly prepared.

(b) *Zinc sulfate.*—Dissolve 1 g of C.P.  $\text{Zn}$  in  $\text{H}_2\text{SO}_4$  and dilute to 1000 ml. 1 ml = 0.001 g of  $\text{Zn}$ .

23

## DETERMINATION

Transfer aliquot equivalent to 25 g of plant material from stock soln (filtrate from  $\text{Cu}$  determination—last paragraph under 21) to 250 ml Erlenmeyer flask and add  $\text{NH}_4\text{OH}$  in slight excess. Dissolve precipitate in slight excess of pure glacial acetic acid, saturate soln with  $\text{H}_2\text{S}$ , and set aside several hours for precipitate to

settle. The acidity must be kept between pH 2 and pH 3, and the presence of a citrate helps to prevent precipitation of Fe and Mn. Hence at this point add ca 2 g of citric acid, ammonia until neutral to methyl orange, and 10 ml of a formic acid soln (containing in 100 ml of mixture, 3 ml of  $\text{NH}_4\text{OH}$ , 20 ml of 90% formic acid, and 25 g of  $(\text{NH}_4)_2\text{SO}_4$ ). Filter on pad of paper pulp, 20(c), and wash with soln of acetic acid (1+10) containing 10 g of  $\text{NH}_4$  acetate in 100 ml of soln, saturated with  $\text{H}_2\text{S}$ . Ignite pad of paper pulp and precipitate in porcelain crucible, cool, dissolve residue in a few drops of  $\text{HCl}$  (1+9), and warm on hot water bath. Transfer to 100 ml beaker and add  $\text{NH}_4\text{OH}$  in slight excess. Heat on water bath 5 min., filter, and wash precipitate. Add acetic acid in slight excess to filtrate and saturate the hot soln with  $\text{H}_2\text{S}$ ; stopper tightly and set aside several hours in warm place for precipitate to settle. Filter, wash as previously directed and ignite in porcelain crucible. Dissolve ignited residue of  $\text{ZnO}$  in 10 ml of 0.1  $N$   $\text{H}_2\text{SO}_4$ , make to volume of 50 ml, and mix. Transfer aliquot of 5 ml to 50 ml Nessler tube containing 5 ml of the K ferro-cyanide. Dilute to 50 ml, mix with stirring rod, and let stand 5–10 min. To another Nessler tube containing 5 ml of the K ferro-cyanide diluted to ca 40 ml, add dropwise with stirring the Zn sulfate until turbidity of standard matches turbidity of sample. From number of ml of Zn standard required calculate percentage of Zn.

#### ARSENIC—TENTATIVE

24

PREPARATION OF SOLUTION.—See XXIX, 3.

25

#### DETERMINATION

Proceed as directed under XXIX, 4 and 5, or take aliquot and determine as directed under VI, 12, beginning "add 3 ml of  $\text{H}_2\text{SO}_4$ ."

#### SULFUR

##### *Sodium Peroxide Method<sup>11</sup>—Official*

26

#### PREPARATION OF SOLUTION

Place 1.5–2.5 g of material in Ni crucible of ca 100 ml capacity and add 5 g of anhydrous  $\text{Na}_2\text{CO}_3$ . Mix thoroly, using Ni or Pt rod, and moisten with ca 2 ml of  $\text{H}_2\text{O}$ . Add  $\text{Na}_2\text{O}_2$ , ca 0.5 g at a time, thoroly mixing charge after each addition, and continue until mixture becomes nearly dry and quite granular (ca 5 g of  $\text{Na}_2\text{O}_2$  required). Place crucible over a S-free flame or electric hot plate and heat carefully, with occasional stirring until contents are fused. (If material ignites, determination is worthless.) After fusion, remove crucible, allow to cool somewhat, and cover hardened mass with more of the  $\text{Na}_2\text{O}_2$  to depth of ca 0.5 cm. Heat gradually and finally with full flame until fusion again takes place, rotating crucible from time to time in order to bring any particles adhering to sides into contact with oxidizing material. Continue heating 10 min. after fusion is complete. Cool somewhat, place warm crucible and contents in 600 ml beaker, and carefully add ca 100 ml of  $\text{H}_2\text{O}$ . After initial violent action has ceased, wash material out of crucible, make slightly acid with  $\text{HCl}$  (adding small portions at a time), transfer to 500 ml flask, cool, and dilute to volume. Filter, and determine sulfates in an aliquot of filtrate as directed under 27.

27

#### DETERMINATION

Add  $\text{H}_2\text{O}$  to make aliquot to ca 200 ml and add  $\text{HCl}$  to make ca 0.5 ml of free acid present. Heat to boiling and add 10 ml of 10%  $\text{BaCl}_2$  soln dropwise with constant stirring. Continue boiling ca 5 min. and allow to stand 5 hours or longer in warm

place. Decant liquid thru ashless filter or ignited and weighed Gooch crucible, treat precipitate with 15–20 ml of boiling  $H_2O$ , transfer to filter, and wash with boiling  $H_2O$  until filtrate is free from chlorides. Dry precipitate and filter, ignite, and weigh as  $BaSO_4$ . Multiply result by factor 0.13736 and report as percentage of S.

*Magnesium Nitrate Method*<sup>12</sup>—Official

28

PREPARATION OF SOLUTION

Weigh 1–5 g of material into large porcelain or Sillimanite crucible. Add 7.5 ml of  $Mg(NO_3)_2$  soln, II, 7(e), taking care that all material is brought in contact with soln, and heat on electric hot plate ( $180^\circ$ ) until no further action takes place. Transfer crucible while hot to electric muffle and allow it to remain at low heat (muffle must not show any red) until charge is thoroly oxidized. (No black particles should remain. It may be necessary to break up charge and return to muffle.) Remove crucible from muffle and allow to cool. Add  $H_2O$ , then  $HCl$  in excess. Bring soln to boil, filter, and wash thoroly. If preferred, transfer soln to 250 ml volumetric flask before filtering and make to mark with  $H_2O$ .

29

DETERMINATION

Dilute entire filtered soln, 28, to 200 ml or take an aliquot of 100 ml of the measured volume, make to 200 ml, and proceed as directed under 27.

PHOSPHORUS<sup>13</sup>

30

*Macro Method*—Official

(a) *For samples exceedingly high in P and low in Ca and Mg, such as certain seeds, grains, etc.*—Prepare as directed under 28, or evaporate filtrate and washings from S determination, 27, to 50 ml and proceed as directed under II, 9 or 12.

(b) *For all other samples.*—Take 50 ml aliquot of Soln A, under 5, and proceed as directed under II, 9 or 12.

*Micro Method*<sup>14</sup>—Official

31

REAGENTS

(a) *Standard potassium dihydrogen phosphate soln.*—Dissolve 0.4394 g of pure dry  $KH_2PO_4$  in  $H_2O$  and make up to liter. 50 ml of this soln diluted to 200 ml gives a standard of which 2 ml = 0.05 mg of P.

(b) *Ammonium molybdate soln.*—Dissolve 25 g of  $NH_4$  molybdate in 300 ml of  $H_2O$ . Dilute 75 ml of  $H_2SO_4$  to 200 ml and add to the  $NH_4$  molybdate soln.

(c) *Hydroquinone soln.*—Dissolve 0.5 g of hydroquinone in 100 ml of  $H_2O$ , and add one drop of  $H_2SO_4$  to retard oxidation.

(d) *Sodium sulfite soln.*—Dissolve 200 g of  $Na_2SO_3$  in  $H_2O$ , make up to a liter, and filter. Either keep this soln well-stoppered or make it up fresh each time.

(e) *Magnesium nitrate soln.*—Dissolve 160 g of  $MgO$  in  $HNO_3$  (1+1), avoiding excess of the acid; add a little  $MgO$  in excess, boil, filter from the excess  $MgO$ ,  $Fe_2O_3$ , etc., and dilute to 1 liter.

32

PREPARATION OF SOLUTION

To 1 or 2 g of substance in small Sillimanite crucible add 1 ml of the  $Mg(NO_3)_2$  soln, and place on steam bath. After a few minutes cautiously add a few drops of  $HCl$ , taking care that formation of gas bubbles does not push portions of sample over edge of crucible. Make 2 or 3 further additions of a few drops of  $HCl$  while



sample is on bath so that as it approaches dryness there is a tendency for it to char. If contents of crucible become so viscous that no further drying may be obtained on bath, complete drying on hot plate, put on crucible cover, transfer to cold muffle, and ignite at dull red heat 6 hours, or until an even grey ash is obtained. (It may be necessary to cool crucible, dissolve ash in a little  $\text{H}_2\text{O}$  or alcoholic glycerol, evaporate to dryness, and return uncovered to muffle for 4–5 hours longer.) Cool, take up with  $\text{HCl}$  (1+4), and transfer to 100 ml beaker. Add 5 ml of  $\text{HCl}$  and evaporate to dryness on steam bath to dehydrate  $\text{SiO}_2$ . Moisten residue with 2 ml of  $\text{HCl}$ , add ca 50 ml of  $\text{H}_2\text{O}$ , heat a few minutes on bath, transfer to 100 ml volumetric flask, cool immediately, make to volume, and filter, discarding first portion of filtrate.

## 33

## DETERMINATION

To 5 ml aliquot of filtrate in 10 ml volumetric flask add 1 ml of the  $\text{NH}_4$  molybdate, rotate flask to mix, and allow to stand a few moments. Add 1 ml of the hydroquinone soln, again rotate flask, and add 1 ml of the  $\text{Na}_2\text{SO}_3$  soln. (These last 3 additions may be made with a Mohr pipet.) Make to volume with  $\text{H}_2\text{O}$ , stopper mouth of flask with thumb or forefinger, and shake to mix contents thoroly. Allow to stand 30 min. and compare immediately in a colorimeter with 2 ml of the standard  $\text{KH}_2\text{PO}_4$  soln treated simultaneously and in identical manner. (With either unknown or standard set at 25.0 mm, readings within 10 mm, *i.e.*, a range of 20 mm, are accurate. If concentration of P in unknown set is outside this range, it may be brought nearer to that of standard by diluting filtrate, ashing a smaller or larger sample, making filtrate to smaller or larger volume, or using smaller aliquot.) Report as percentage of P.

CHLORINE<sup>15</sup>

(If bromides or iodides are present in significant quantities results must be corrected accordingly.)

## 34

## PREPARATION OF SOLUTION—OFFICIAL

First verify complete retention of Cl in each kind of material by trial since losses can occur, especially with samples high in carbohydrates, if insufficient  $\text{Na}_2\text{CO}_3$  is present during ignition, or in any case if excessive temps. are used.

Moisten 5 g of substance in Pt dish with 20 ml of a 5%  $\text{Na}_2\text{CO}_3$  soln, evaporate to dryness, and ignite as thoroly as possible at a temp. not exceeding dull redness. Extract with hot  $\text{H}_2\text{O}$ , filter, and wash. Return residue to Pt dish and ignite to ash; dissolve in  $\text{HNO}_3$  (1+4), filter from any insoluble residue, wash thoroly, and add this soln to  $\text{H}_2\text{O}$  extract.

## 35

*Gravimetric Method—Official*

To prepared soln, 34, add 10%  $\text{AgNO}_3$  soln, avoiding more than slight excess. Heat to boiling, protect from light, and allow to stand until precipitate is coagulated. Filter on weighed Gooch crucible, previously heated to 140–150°, and wash with hot  $\text{H}_2\text{O}$ , testing filtrate to prove excess of  $\text{AgNO}_3$ . Dry the  $\text{AgCl}$  at 140–150°, cool, and weigh. Report as percentage of Cl.

*Volumetric Method I<sup>16</sup>—Official*

(Limit of accuracy of this titration is considered to be  $\pm 0.2$  mg of Cl, hence an accuracy of 1.0% would require samples containing not less than 20 mg.)

36

## REAGENTS

(a) *Silver nitrate soln.*—Adjust to exact 0.1 *N* strength by standardizing against a 0.1 *N* NaCl soln containing 5.846 g of pure NaCl per liter.

(b) *Ammonium or potassium thiocyanate.*—0.1 *N*. Adjust by titrating against the 0.1 *N* AgNO<sub>3</sub>.

(c) *Ferric indicator.*—A saturated soln of ferric ammonium alum.

(d) *Nitric acid.*—Free from lower oxides of N by diluting the usual pure acid with ca  $\frac{1}{4}$  volume of H<sub>2</sub>O, and boiling until perfectly colorless.

37

## DETERMINATION

To the prepared soln, 34, add a known volume of the AgNO<sub>3</sub> in slight excess. Stir well, filter, and wash AgCl precipitate thoroly. To combined filtrate and washings add 5 ml of the ferric indicator and a few ml of the HNO<sub>3</sub> and titrate excess of Ag with the thiocyanate until a permanent light brown color appears. From number of ml of AgNO<sub>3</sub> used, calculate quantity of Cl. 1 ml of 0.1 *N* AgNO<sub>3</sub> = 0.00355 g of Cl.

*Volumetric Method II<sup>17</sup>—Official*

38

## REAGENTS

(a) *Standard potassium iodide soln.*—Weigh out 4.6822 g of the pure (A.C.S. spec.) salt, dried to constant weight at 105–150°, dissolve in H<sub>2</sub>O, and dilute to 1 liter. 1 ml = 1 mg of Cl.

(b) *Silver nitrate soln.*—Approximately 0.3 *N*. Dissolve 48 g of the salt in H<sub>2</sub>O, filter, and dilute to 1 liter. 1 ml = 10 mg of Cl (ca).

(c) *Standard silver nitrate soln.*—Dilute 100 ml of Reagent (b) to ca 900 ml and adjust by standardizing against Reagent (a) so that 1 ml = 1 mg of Cl.

(d) *Chlorine-free starch indicator.*—For each 100 ml of final soln take 2.5 g of soluble starch and make to a paste with cold H<sub>2</sub>O. After stirring out lumps, add 25–50 ml more cold H<sub>2</sub>O and stir or shake 5 min. Centrifuge, decant, and discard liquid. Repeat extraction 3 times and finally transfer residue to flask containing proper quantity of boiling H<sub>2</sub>O. Stir again, allow to come to boil, cover with small beaker, and cool under tap, shaking occasionally.

(e) *Dilute sulfuric acid soln.*—Add 35 ml of H<sub>2</sub>SO<sub>4</sub> to each liter of H<sub>2</sub>O, boil 5–10 min., and cool to room temp.

(f) *Iodine soln.*—Shake a large excess of I crystals in glass-stoppered bottle nearly filled with Reagent (e). Decant, and discard soln. Repeat process but decant soln into glass-stoppered bottle. Test soln by adding 25 ml of it to 25 ml of Reagent (e), followed by 5 ml of Reagent (d). (No blue color should appear after 5 min., and the color produced by small amount of Reagent (a) should be discharged by an equivalent amount of Reagent (c).) If soln gives blue color when tested, compute quantity of Reagent (c) needed to treat remainder of decanted soln from excess of Reagent (c) over Reagent (a) observed in test titrations. Add twice that amount and test as before.

(g) *Potassium permanganate.*—Dissolve 60 g of the salt in 400 ml of warm H<sub>2</sub>O (ca 50°) and dilute to 1 liter.

(h) *Potassium sulfate—copper sulfate mixture.*—Thoroly mix 16 parts of K<sub>2</sub>SO<sub>4</sub> with 1 part of CuSO<sub>4</sub>·5H<sub>2</sub>O.

(i) *Wash soln.*—Mix 980 ml of H<sub>2</sub>O with 20 ml of HNO<sub>3</sub>.

39

## DETERMINATION

Weigh into beaker such a quantity of sample as is expected to contain 10–40

mg of Cl. (If more than 4 g is taken, use proportionately more  $\text{HNO}_3$  and  $\text{KMnO}_4$  soln.) Add 10 ml of the 0.3 N  $\text{AgNO}_3$  soln and stir until sample is thoroly soaked with the soln, adding a little  $\text{H}_2\text{O}$  or warming if necessary. Add 25 ml of  $\text{HNO}_3$ , stir, add 5 ml of the  $\text{KMnO}_4$  soln, and stir until frothing ceases. Place mixture in water bath or on hot plate to keep it just below boiling. Stir, and wash down sides of beaker at intervals with least possible quantity of  $\text{H}_2\text{O}$ . After 20 min., or when there appears to be no further action on sample, add more of the  $\text{KMnO}_4$  soln, a little at a time, until color begins to fade slowly. Dilute to ca 125 ml with boiling  $\text{H}_2\text{O}$  and heat 10 min. longer. (Beaker may stand in bath or on hot plate until ready to filter.) Filter while hot thru Whatman's No. 5, or similar paper, with suction as follows: Place disk of 30-mesh stainless steel wire gauze or of No. 40 filter cloth in bottom of 3" Hirsch funnel. Fold 9 cm paper over bottom of No. 11 rubber stopper, shaping it to funnel by making 9-10 folds up side of stopper. Place paper in funnel and apply strong suction. Wet paper and keep it wet while fitting it into funnel so as to avoid double thicknesses of paper. Thoroly wash paper, first with  $\text{H}_2\text{O}$ , then with the wash soln. Discard washings and rinse out flask. Pour supernatant liquid thru filter and transfer precipitate and sample residue to filter. If filtrate is not turbid, or if it is only slightly opalescent, wash precipitate thoroly, applying wash soln very gently, but maintaining strong suction on filter. If combined filtrate and washings are clear, test them for Ag. If turbid, re-heat and pass thru filter, repeating until clear, and finally wash as directed above. If filtrate does not give a definite test for Ag, repeat determination on fresh but smaller portion of sample. Place filter paper and contents in Kjeldahl flask and add such quantities of mixture of  $\text{CuSO}_4$  and  $\text{K}_2\text{SO}_4$  and of  $\text{H}_2\text{SO}_4$  as would be appropriate for a protein determination on same kind and amount of material and digest in similar manner. (For 2 g of grass, 8 g of the sulfate mixture and 20 ml of acid are enough.) When digest is cool, add 175 ml of  $\text{H}_2\text{O}$ , boil 5-10 min., and cool to room temp. Titrate the  $\text{Ag}_2\text{SO}_4$  in the Kjeldahl flask with the standard KI, using 5 ml of starch and 30 ml of the I soln. (Latter is added just before titration.) Wash down neck of flask after each addition of KI when near end point and titrate until blue color persists after shaking. If less than 30 mg of Cl is present, add the starch and I soln at beginning. If a larger but unknown amount is present, add 2 ml of starch and 10 ml of I soln at beginning and titrate until approach of end point is seen. Shake vigorously to coagulate precipitate, add remainder of starch and I and proceed to end point. If a known large amount is present, titrate to within 2 ml of end point, shake as above, add indicator reagents, and continue titration. If end point is over-run, add 5 ml of the standard  $\text{AgNO}_3$  and titrate again.

Blank determinations are not necessary after reagents have been tested. If blanks made by using pure sugar as a sample exceed 0.05 mg, examine filter paper and various reagents carefully.

## 40

IODINE<sup>18</sup>—TENTATIVE

Weigh sample of 50 g of finely ground, air-dried plant material; transfer to porcelain dish, and thoroly mix with 10 g of finely pulverized  $\text{CaO}$  and 10 g of finely pulverized  $\text{CuO}$ . Distribute mixture in 3 alundum boats and place end to end in a large combustion tube. Close right end of combustion tube tightly with rubber stopper that carries glass tube connecting with wash bottle. Connect wash bottle on left to suction pump, and close electric circuit. When tube 3 attains red heat draw air thru system and light first burner on left of gas furnace. (After short time heat from this burner sets plant material in first boat on fire, and a moderately rapid current of air drawn thru the tubes and heat from the other burners of gas furnace lighted at proper time keep sample burning at slow and uniform rate, some-

what in manner of a lighted cigar. Any unburned vapors from sample are drawn over the red-hot platinized asbestos catalyst, where they are completely burned, and the I vapors are carried into the gas wash bottles and absorbed.)

After combustion is completed, turn off heat and cool apparatus by continuing to draw a current of air thru system. Disconnect suction pump and remove boats carefully. Digest ash, leach with hot  $H_2O$ , and combine filtrate from ash with  $K_2CO_3$  solns from absorption flasks and evaporate to dryness.

Add just enough  $H_2O$  to dissolve residue and transfer soln to separator of proper size. Add enough 95% pure ethyl alcohol to form two immiscible layers and shake separator vigorously ca 10 min. Run aqueous portion of soln into another separator, and repeat process of extraction 3 times. Combine alcoholic extracts that contain the I and evaporate slowly in small silica dish to dryness, avoiding spattering. Heat in electric furnace having pyrometer attachment 30 min. at  $400^\circ$  to char any organic matter present.

Cool, dissolve residue in a few drops of  $H_2O$ , filter into small separator, and make slightly acid with  $H_2SO_4$  (1+3). Add ca 3 ml of saturated soln of sulfurous acid, stopper separator, and shake vigorously ca 1 min. to reduce iodate, if present, to iodide. Add 1 ml of pure  $CS_2$ , accurately measured, and ca 2 ml of 10% soln of I-free  $NaNO_2$ . Stopper funnel and shake vigorously ca 1 min. and allow the  $CS_2$  to settle. (If in the  $CS_2$  slight pink color is evident, all the I has been absorbed.) If the  $CS_2$  has a deep pink color, run it into a centrifuge tube, add 1 ml of  $CS_2$ , and repeat extraction until last portion has only a faint color. Combine extracts, centrifuge, and compare a portion in micro-colorimeter with an I standard prepared similarly. Report results in p.p.m. if high, or p.p.b. if low.

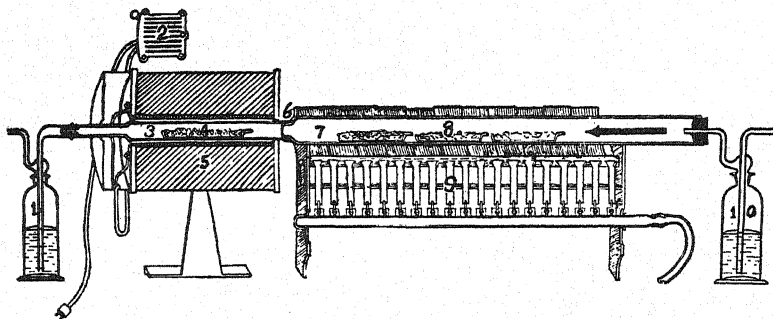


FIG. 17.—APPARATUS FOR DETERMINATION OF IODINE

1. Absorption bottle containing 5%  $K_2CO_3$  (2 bottles used); 2. rheostat; 3. silica catalyst tube; 4. platinized asbestos catalyst; 5. electric tube furnace, maximum temp.  $1100^\circ$ ; 6. asbestos cement seal, sealing large combustion tube to smaller tube containing catalyst; 7. silica combustion tube; 8. alundum boats containing sample; 9. gas combustion furnace; and 10. wash bottle containing 10%  $K_2CO_3$ .

41

#### SELENIUM<sup>19</sup>

(Applicable to materials containing over 2 p.p.m. of selenium.)

Grind air-dried sample and carefully prepare uniform subsample. Prepare mixture of 50 ml of  $H_2SO_4$  and 100 ml of 68%  $HNO_3$  in 600 ml beaker. Add 5 g of powdered sample to acid mixture slowly, with stirring, restricting temp. of mixture to not above  $80^\circ$ . After adding sample when first vigorous reaction is over, warm gently

with occasional stirring until fumes of nitrogen peroxide cease to evolve. Warm at temp. not to exceed 120° until slight darkening of liquid begins to appear. Transfer liquid to distilling apparatus and proceed as directed in I, 26.

SUGARS<sup>20</sup>

42

## PREPARATION OF SOLUTION—OFFICIAL, FIRST ACTION

(a) *Extraction*.—Prepare sample as described under 2(b). Pour alcoholic soln thru filter paper or extraction thimble, catching filtrate in volumetric flask. Transfer insoluble material to beaker, cover with 80% alcohol, warm on steam bath 1 hour, allow to cool, and again pour the alcoholic soln thru same filter. If second filtrate is highly colored, repeat extraction. Transfer residue to filter, allow to drain, and dry. Grind residue so that all particles will pass thru a 1-mm sieve, transfer to extraction thimble, and extract 12 hours in Soxhlet apparatus with 80% alcohol. Dry residue and save for starch determination. Combine alcoholic filtrates and make to volume at a definite temp. with 80% alcohol.

(b) *Clearing*.—Place an aliquot of the alcoholic extract in beaker on steam bath and drive off alcohol. Avoid evaporation to dryness by adding H<sub>2</sub>O if necessary. When odor of alcohol has disappeared from sample, add ca 100 ml of H<sub>2</sub>O and heat to 80° to soften gummy precipitates and break up insoluble masses. Cool to room temp. and proceed as directed under (1) or (2).

(1) Transfer soln to volumetric flask and rinse beaker thoroly with H<sub>2</sub>O, adding rinsings to contents of flask. Add enough saturated neutral Pb acetate to produce a flocculent precipitate, shake thoroly, and allow to stand 15 min. Test supernatant liquid with a few drops of saturated Pb acetate. If more precipitate forms, shake, and allow to stand again; if no further precipitate forms, dilute to mark with H<sub>2</sub>O, mix thoroly, and filter thru dry filter. Add sufficient solid Na oxalate to filtrate to precipitate all the Pb, and refilter thru dry paper. Test filtrate for presence of Pb with a little solid Na oxalate.

(2) Add double minimum amount of saturated neutral Pb acetate soln that is required to cause complete precipitation, as found by testing a portion of supernatant liquid with a few drops of dilute Na oxalate soln. After allowing mixture to stand a few minutes only, filter immediately into beaker to which has been added an estimated excess of Na oxalate crystals. Allow Pb precipitate to drain on filter and wash with cold H<sub>2</sub>O until filtrate no longer gives a precipitate in the oxalate soln. Excess of oxalate must be assured by testing with a drop of Pb acetate soln. Filter off and wash precipitated Pb oxalate, catching filtrate and washings in volumetric flask. Dilute to mark with H<sub>2</sub>O and mix.

## REDUCING SUGARS

43

*Munson and Walker General Method—Tentative.—See XXXIV, 37.*

*Quisumbing and Thomas Method<sup>21</sup>—Official, First Action*

44

## REAGENTS

(a) *Copper sulfate soln*.—Wash crystals of C.P. CuSO<sub>4</sub> · 5H<sub>2</sub>O free from dust, etc., with H<sub>2</sub>O, dissolve in hot H<sub>2</sub>O to make a saturated soln, and filter. Determine Cu electrolytically and dilute soln so that 25 ml of it will contain 525 mg of Cu (41.2 g of CuSO<sub>4</sub> · 5H<sub>2</sub>O in 500 ml of soln).

(b) *Alkaline tartrate soln*.—Prepare saturated soln of NaOH (purified by alcohol) and let stand several days, until insoluble carbonates and other impurities have settled out. Siphon off clear soln and establish its alkalinity by titration with stand-

ard acid. Dissolve 173 g of highest purity Rochelle salts in  $\text{H}_2\text{O}$  in 500 ml graduated flask and add calculated quantity of  $\text{NaOH}$  soln so that 500 ml of this alkaline tartrate soln will contain exactly 65 g of  $\text{NaOH}$ . Make to mark with  $\text{H}_2\text{O}$ .

45

## DETERMINATION

Measure exactly 25 ml each of the  $\text{CuSO}_4$  and alkaline tartrate solns into 400 ml Pyrex or Bohemian glass beaker, the diameter of which is ca 9 cm. Add 50 ml of sugar soln containing preferably 50–150 mg of sugar. Cover beaker with watch-glass and place beaker in water bath maintained at  $80^\circ$ . After digesting exactly 30 min., filter the  $\text{Cu}_2\text{O}$  by suction thru mat of asbestos in Gooch crucible. Wash precipitate with  $\text{H}_2\text{O}$ . Determine Cu by one of methods below. Calculate weight of sugar from tables of Quisumbing and Thomas (XLIII, 17).

46

(a) *Direct Weighing of Cuprous Oxide—Official, First Action.—See XXXIV, 39.*

47

(b) *Volumetric Permanganate Method<sup>22</sup>—Tentative*

Filter and wash the  $\text{Cu}_2\text{O}$  as directed under 45. With aid of stirring rod transfer asbestos mat and  $\text{Cu}_2\text{O}$  back into beaker in which reduction took place. Rinse inside of crucible and lip of beaker with 10 ml of a soln of 240.9 g of crystalline ferric  $(\text{NH}_4)_2\text{SO}_4$  and 200 ml of  $\text{H}_2\text{SO}_4$  dissolved in  $\text{H}_2\text{O}$  and made up to 1 liter. Cool the dilute  $\text{H}_2\text{SO}_4$  before adding the salt. Receive rinsings in beaker containing the  $\text{Cu}_2\text{O}$ . Holding crucible over beaker, stir contents of beaker thoroly with stirring rod until the  $\text{Cu}_2\text{O}$  has gone into soln. Wash crucible with ca 25 ml of hot  $\text{H}_2\text{O}$  ( $80^\circ$ ), receiving washings in beaker. Stir contents of beaker and then raise beaker to see if any undissolved particles of  $\text{Cu}_2\text{O}$  are resting on bottom. If so, press out each one with point of stirring rod until all have gone into soln. Add ca 125 ml more hot  $\text{H}_2\text{O}$ . Add 1 drop of a soln of 0.15 g of orthophenanthroline monohydrate and 0.07 g of  $\text{FeSO}_4$  in 10 ml of  $\text{H}_2\text{O}$ . Titrate at once with continual stirring with 0.05 N  $\text{KMnO}_4$ . (In a long titration it is best to add indicator just before end point is reached.) Standardize the  $\text{KMnO}_4$  as directed in XLII, 14 and 15.

48

## SUCROSE—TENTATIVE

(a) *Hydrochloric Acid Inversion*

Using aliquot of cleared soln obtained in 42, proceed as directed under XXVII, 29.

(b) *Invertase Inversion*

(1) *For plants giving an end point of hydrolysis within 2 hours.*—Pipet aliquot of cleared soln obtained in 42 into beaker in which reduction is to take place. Make slightly acid to methyl red with acetic acid. Add 3 drops of 1% soln of Wallerstein's red label invertase. Let mixture stand at room temp. 2 hours. Add reagents as directed in 44 or in XXXIV, 37 and determine reducing power. Calculate results as invert sugar. Deduct reducing power of original soln, also expressed as invert sugar, and multiply difference by factor 0.95.

(2) *For plants giving a slower end point of hydrolysis.*—Place aliquot of soln obtained in 42 in small volumetric flask. Make slightly acid to methyl red with acetic acid. Add 3 drops of 1% soln of Wallerstein's red label invertase and a few drops of toluene. Stopper flask and let stand overnight or longer at room temp. Dilute to mark with  $\text{H}_2\text{O}$  and use aliquot for reducing power as directed above. Results may include some other carbohydrates slowly hydrolyzed by invertase.

49

ETHER EXTRACT—TENTATIVE.—See XXVII, 22.

50

CRUDE FIBER—TENTATIVE.—See XXVII, 27.

51

TOTAL NITROGEN.—See II, 25.

AMMONIA IN TOBACCO<sup>23</sup>—TENTATIVE

52

## REAGENTS

(a) *Ammonium sulfate stock soln.*—Dissolve 2.358 g of pure salt in H<sub>2</sub>O and make up to 1000 ml. 2 ml = 1.0 mg of N. Preserve by adding a few drops of CHCl<sub>3</sub>.

(b) *Ammonium sulfate standard soln.*—Dilute 200 ml of (a) to 1000 ml. 1 ml = 0.1 mg of N. Preserve with CHCl<sub>3</sub>.

(c) *Nessler's soln* (Folin\*).—Transfer 37.5 g of KI and 27.5 g of I to 250 ml flask, and add 25.0 ml of H<sub>2</sub>O and 35–40 g of Hg. Shake flask continuously and vigorously 7–15 min., or until nearly all dissolved I has disappeared. (The soln becomes hot.) When the red I soln has begun to pale visibly, though still red, cool in running H<sub>2</sub>O, and continue shaking until reddish color of I has been replaced by greenish color of double iodide. (Whole operation should not take more than 15 min.) Separate soln from surplus Hg by decantation and washing with liberal quantities of H<sub>2</sub>O. Dilute soln and washings to 500 ml. (If cooling was begun in time, the resulting concentrated soln of double iodide is clear enough for immediate dilution with 10% NaOH and H<sub>2</sub>O and the finished Nessler's reagent can be used at once.) Place 700 ml of 10% NaOH soln in liter flask, add 150 ml of the clear concentrated soln of the double iodide, mix, and dilute to 1 liter with H<sub>2</sub>O. Allow to settle if turbidity develops.

(d) *Permutit* (Folin\*).—Pass thru sieves and reject material smaller than 80-mesh and larger than 60-mesh. Wash copiously with H<sub>2</sub>O by decantation until the whole settles rapidly and contributes no more dust or turbidity to the H<sub>2</sub>O. Dry in current of air in thin layer without heating. For recovery of permutit after use, see Folin's manual.\*

53

## DETERMINATION

Transfer an accurately weighed 0.5 g sample of dry finely powdered tobacco to 300 ml Kjeldahl flask; add 25–30 ml of H<sub>2</sub>O, a small piece of paraffin, a few angular quartz pebbles, and 2–2.5 g of light MgO. Prepare stopper to fit Kjeldahl flask with a piece of 9 mm outside diameter glass tubing bent around thru 180°, the short limb of bend inserted thru stopper and the longer limb reaching to level of desk as flask is held in clamp over micro burner. (Or, if more convenient, cut longer limb and join again by short length of rubber tubing at point ca 15 cm from lower end.) Connect distillation tube so prepared to flask and dip lower end into a short wide test tube (50 ml centrifuge tube) that contains 5 ml of 0.1 N HCl and a few drops of methyl red indicator, II, 19(i). Heat contents of flask with micro burner at such a rate that steam begins to rise from receiver in ca 3 min. Make no effort to cool distillation tube or receiver. Distil 5 min., counting time from point at which distillate first runs down tube. Remove tube and wash end into receiver with a few drops of H<sub>2</sub>O. Cool distillate and dilute to 50 ml. Charge several 100 ml volumetric flasks with 2.5–3.0 g of the washed and dried permutit and wash each several times by decantation with H<sub>2</sub>O. Transfer to 3 of the flasks 3 ml of 0.1 N HCl and portions of the standard (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> soln (b) containing 0.3, 0.5, and 1.0 mg of N, respectively. Add sufficient H<sub>2</sub>O to each flask to make total volume of 25 ml. Transfer

\* Laboratory Manual of Biological Chemistry, New York, 4th ed., p. 293 (1926). The reagent prepared by usual procedure is, however, equally satisfactory.

25 ml aliquot of distillate from each determination to flask containing permutit. Shake all flasks 5 min. with a gentle rotatory motion and lay them on their sides on suitable support 1 min. Decant fluid from each flask and wash permutit by decantation 3 times successively with 10–30 ml of  $H_2O$ , allowing soln to settle 1 min. before each decantation. Rinse permutit to bottom of each flask with 5 ml of  $H_2O$ , add 1 ml of 10%  $NaOH$  soln, and rotate 3 min. Add 65 ml of  $H_2O$ , rotate, and add 10 ml of the Nessler reagent. Dilute to mark, mix, and in colorimeter compare color of soln derived from each determination with known standard that most nearly matches it. (Color is stable several hours.) Report  $NH_3$  nitrogen as percentage of sample of tobacco used.

FREE NICOTINE IN TOBACCO<sup>22</sup>—TENTATIVE

54

## DETERMINATION

Mix ca 2.5 g of dry powdered tobacco with 50 ml of  $H_2O$ . Stir 5–10 min., allow to settle, and decant necessary quantity into the cell of quinhydrone or hydrogen electrode. Determine pH value with accuracy of 0.1 unit. Construct a curve by plotting data in following table on conveniently large scale. Read percentage of free nicotine from this curve at a point corresponding to pH found and report as percentage of total nicotine in free form.

55	FREE NICOTINE per cent	pH	FREE NICOTINE per cent	pH
	1	6.11	50	8.11
	2	6.42	55	8.20
	5	6.86	60	8.29
	10	7.15	65	8.37
	15	7.36	70	8.48
	20	7.51	75	8.59
	25	7.63	80	8.71
	30	7.74	85	8.86
	35	7.85	90	9.06
	40	7.93	95	9.39
	45	8.02		

NITRATE NITROGEN<sup>24</sup>—TENTATIVE

(Applicable to tobacco and other plant tissues.)

56

## REAGENTS

(a) *Sulfuric acid soln.*—4 *N*. Prepare from C.P. special reagent low in N.  
 (b) *Sulfuric acid soln.*—18 *N*. Prepare from C.P. special reagent low in N.  
 (c) *Reduced iron powder.*—Determine titration value of the  $NH_3$  in the powder by boiling 3.0 g of it with 50 ml of the 4 *N*  $H_2SO_4$  5 min., cooling, making alkaline with  $NaOH$ , distilling into 0.1 *N* acid, and titrating with 0.1 *N* alkali to methyl red. Divide by 10 to obtain correction to be used with the 0.3 g of powder used in method.

(d) *Ammonium sulfate stock soln.*—Dissolve 2.358 g of pure salt in  $H_2O$  and make up to 1000 ml. 2 ml = 1.0 mg of N. Add no preservative.

(e) *Ammonium sulfate standard soln.*—Dilute 200 ml of (d) to 1000 ml. 1 ml = 0.1 mg of N.

(f) *Diphenylamine soln.*—Suspend 0.5 g of diphenylamine in 20 ml of  $H_2O$  and add  $H_2SO_4$  to make 100 ml. Cool, and preserve in dark bottle.

57

## DETERMINATION

Ascertain quantity of the 4 *N*  $H_2SO_4$  required to bring a 2 g sample of the dried



and powdered tissue to ca pH 1.0 as follows: Weigh out 0.5 g, stir in small beaker with 1 ml of the 4 *N* acid, add enough H<sub>2</sub>O to make a thin paste that can be transferred to electrode vessel, add quinhydrone, and determine reaction at potentiometer. Make suitable changes in quantity of acid added to a second 0.5 g sample, as suggested by result of first test, and repeat determination. Continue until quantity required to give a reaction in range pH 0.7–0.9 has been found. Multiply this quantity by 4 to obtain amount required by the 2 g sample used for nitrate determination.

Weigh duplicate 2.00 g samples of the powder, mix each in beaker with required quantity of the 4 *N* H<sub>2</sub>SO<sub>4</sub> until a uniform stiff paste is obtained; add 3.5 g of pure asbestos fiber to each and incorporate thoroly. Transfer mixtures to 26×60 mm paper extraction thimbles by means of glass funnel ca 11 cm long, the upper part of which is a cylinder 4.5 cm in diameter, the lower a cylinder 2 cm in diameter. Make the transfer as follows: Support thimble in wire cage hung in mouth of 400 ml conical extraction flask and clamp funnel in position over it so that smaller end extends ca 1 cm into thimble. Push most of asbestos mixture into thimble with glass rod; brush off beaker, funnel, and rod; and wipe off all particles with small piece of surgical cotton. Finally rinse glassware and brush into thimble with alcohol-free ether. Remove funnel and plug end of thimble with the cotton used to wipe apparatus. Place thimble in the siphon tube of ether extraction apparatus (type designed for rubber analysis), thrust short slim glass rod between thimble and glass to hold paper away from glass wall at one side, and suspend siphon tube close under the metal coil condenser of apparatus by means of fine galvanized iron wire. Place 150–200 ml of alcohol-free ether in conical flask. Cut gasket from soft cardboard to fit recess in plate of metal condenser and set condenser, with attached siphon tube, on extraction flask and hold firmly in position by means of 3 spring paper clips.

Place extraction flasks on electric hot plate, add few angular quartz pebbles, and allow extraction to proceed at least 8 hours at siphoning rate of ca 40 times per hour. (If rate is less, correspondingly longer time must be allowed.) To test for completeness of removal of the HNO<sub>3</sub> prepare concentrated H<sub>2</sub>O extract of residue in thimble and overlay 5 ml of the diphenylamine reagent in test tube with a few ml of this extract. (Appearance of blue layer at junction of the two solns indicates that extraction of the HNO<sub>3</sub> by the ether has been incomplete.)

Treat each ether extract with 25 ml of H<sub>2</sub>O, add 2 drops of phenolphthalein, and make faintly alkaline with 0.5 *N* NaOH with continual agitation. Immerse flask in water bath and evaporate off ether very slowly to avoid frothing; make aqueous soln to 100 ml and transfer aliquot (10 ml or more, depending on nitrate content of tissue) to 300 ml Kjeldahl flask. Add 2.5 ml of the 18 *N* H<sub>2</sub>SO<sub>4</sub> and 0.3 g of the reduced iron powder. Boil gently 5 min., cool, add 20 ml of H<sub>2</sub>O and 10 ml of NH<sub>3</sub>-free concentrated NaOH. Immediately fit flask with Folin and Wright distillation tube and distil as directed under 53, into 3 ml of 0.1 *N* HCl contained in test tube. Transfer distillate to 100 ml flask, dilute to ca 60 ml with NH<sub>3</sub>-free H<sub>2</sub>O, add 10 ml of the Nessler soln, agitate, and make to volume. Prepare standard NH<sub>3</sub> solns by pipetting 3–15 ml of the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> standard soln into 100 ml flasks (0.3–1.5 mg NH<sub>3</sub> nitrogen), diluting, add 10 ml of the Nessler soln, agitating, and making to volume. Read color of soln derived from analysis in colorimeter against nearest standard. Calculate quantity of N in aliquot used for reduction, subtract blank for the NH<sub>3</sub> nitrogen found in the 0.3 g of iron powder used, and calculate the nitrate nitrogen in the 2.00 g sample taken. Report as percentage of the dry tissue.

If the nitrate content of the tissue is 0.1% or less, it is desirable to carry out a blank determination on the alkaline soln of the ether extract. To do this proceed as follows:

Transfer aliquot of the alkaline soln equal to that used for the determination to 300 ml Kjeldahl flask, add 2.5 ml of the 18 N  $\text{H}_2\text{SO}_4$ , boil gently 5 min., cool, add 20 ml of  $\text{H}_2\text{O}$  and 10 ml of NaOH soln, II, 19(g), and distil as directed previously. Transfer distillate to 25 ml volumetric flask, dilute to 15 ml, add 2.5 ml of the Nessler soln, agitate, and make to volume. Compare with  $\text{NH}_3$  standards of 0.05–0.10 mg. Deduct quantity of  $\text{NH}_3$  nitrogen found from quantity found after reduction with the iron powder, correct result for blank due to iron powder, and calculate nitrate nitrogen as before.

To determine nitrate content of extracts from plant tissue proceed as follows:

Transfer to evaporating dish aliquot of extract approximately equivalent to 2 g of dry tissue, make neutral to Congo red if necessary, and evaporate to a sirup (must not be evaporated to dryness). Cool, and add the quantity of the 4 N  $\text{H}_2\text{SO}_4$  found by separate experiment to be required to produce reaction in range pH 0.7–0.9; add 3.5 g of asbestos, and mix thoroly. If mixture is too moist to be transferred to extraction thimble, dry it in vacuum desiccator until this can be done. Proceed with extraction as already described.

#### LIGNIN<sup>28</sup>—TENTATIVE

#### 58

##### PREPARATION OF SAMPLE

Grind plant material in mill to pass thru 80-mesh sieve, and dry at 105°. Extract weighed sample (5–10 g) 30 hours in Soxhlet apparatus with alcohol-benzene soln (32 parts by weight of 95% ethanol and 68 parts by weight of benzene). Dry material in oven to free it from the alcohol-benzene soln and place in flask of suitable size. Add  $\text{H}_2\text{O}$  in proportion of 150 ml to 1 g of sample, and boil mixture under reflux condenser 3 hours. Filter mixture while still hot, preferably thru weighed sintered-glass crucible, and transfer extracted material to flask. Add a 1% HCl soln in proportion of 150 ml of acid soln to 1 g of plant material, and boil under reflux condenser 3 hours. Filter mixture while still hot thru the sintered-glass crucible used in previous operation, wash with  $\text{H}_2\text{O}$  until free of acid, dry at 105°, and weigh. Calculate percentage total loss due to successive extraction with the alcohol-benzene soln, hot  $\text{H}_2\text{O}$ , and the 1% HCl. (In substances not especially rich in carbohydrates and proteins, extraction with hot  $\text{H}_2\text{O}$  may be omitted.)

#### 59

##### APPARATUS

The apparatus required is illustrated in Fig. 18. It consists of a bottle (A) having a capacity of 1500 ml and containing ca 500 ml of  $\text{H}_2\text{SO}_4$ . Attached to A by means of a 2-holed rubber stopper is a 250 ml dropping funnel (C), having the lower end of its stem bent as illustrated, containing HCl. By means of stopcock B, allow the HCl to flow into the  $\text{H}_2\text{SO}_4$ , and dry the HCl gas thus generated by passage thru the  $\text{H}_2\text{SO}_4$  in D. The lower end of the stem of C must be close to the bottom of A, as shown in drawing. Place weighed sample and the fuming HCl in Pyrex test tube 300 mm long and 38 mm in diameter (G). By means of the device O, connect G in parallel to two other tubes (G' and G'', see top view), having same dimensions as G, and provide G, G', and G'' with 2-holed rubber stoppers. Thru one hole pass glass tube having right-angled bend nearly to bottom of large test tube (F, F', and F''). Thru other hole insert another tube having right-angled bend, which extends ca 10 mm into the large test tube. K is bottle containing  $\text{H}_2\text{O}$  for absorption of excess HCl gas that passes thru device P and tube J. Regulate flow of HCl gas thru the three large test tubes by means of the stopcocks shown in top view. Place G, G', and G'' in the wooden box L, provided with supports for the tubes and also

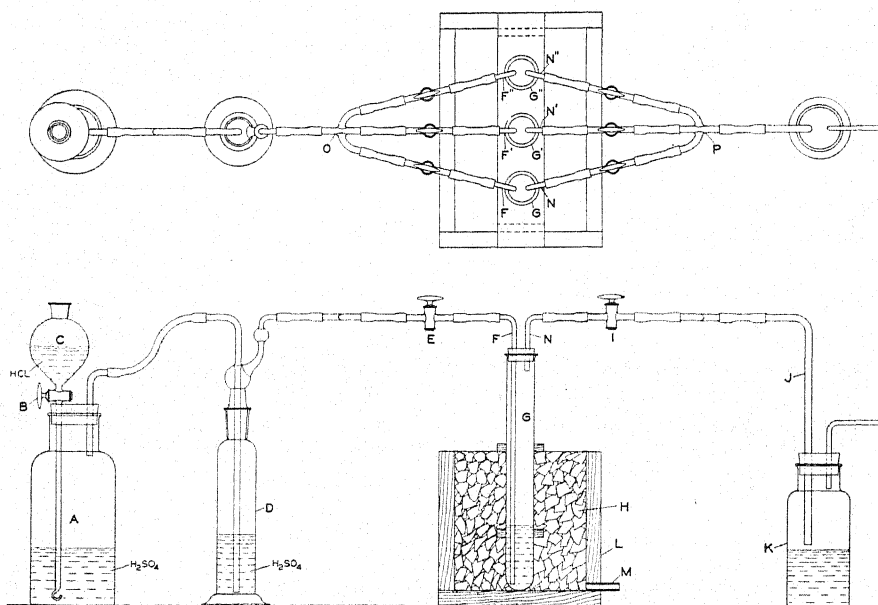


FIG. 18.—APPARATUS FOR DETERMINATION OF LIGNIN

with a drain (M) for removal of  $\text{H}_2\text{O}$ . Surround large test tubes G, G', and G'' in L with crushed ice (H).

## 60

## REAGENT

*Fuming hydrochloric acid.*—Density 1.212–1.223 at  $15^\circ$ . To 500 g of NaCl contained in a liter Pyrex distilling flask provided with ground-glass stopper, add cold soln of 250 ml of  $\text{H}_2\text{O}$  in 450 ml of  $\text{H}_2\text{SO}_4$ . Connect side tube of distilling flask to glass tube, which passes thru a  $\text{H}_2\text{SO}_4$  wash bottle. Connect outlet tube of the  $\text{H}_2\text{SO}_4$  wash bottle to another glass tube, which is immersed in flask containing 3 liters of HCl. Surround flask containing the HCl with crushed ice. Heat distilling flask with small flame and pass the HCl gas into the acid soln until it attains the sp. gr. 1.212–1.223 at  $15^\circ$ . Keep reagent in refrigerating room maintained at  $0^\circ$  or below. (If only a few determinations are to be made, a correspondingly smaller quantity should be prepared.)

## 61

## DETERMINATION

Weigh out three 1 g samples of the extracted and dried material in weighing bottle and place in the three large test tubes, G, G', and G''. Add 20 ml of the reagent to each tube, taking care to wash down with this acid any particles clinging to sides. When all material is wetted with the reagent, add another portion (30 ml). Add ca 3 drops of capryl alcohol to reduce foaming to minimum during subsequent passage of the HCl gas thru reaction mixture. Place the three large test tubes, G, G', and G'', in the wooden box (L) and surround with crushed ice. Lubricate tubes F, F', and F'' with a drop of glycerol so that they move easily thru holes in rubber stoppers. Lead the dry HCl gas from generator into reaction mixtures thru tubes F, F', and F'' (F' and F'' are shown in top view), which reach nearly to bottom of tubes G, G', and G''. Regulate flow of gas thru reaction mixtures in G, G', and G''

by means of stopcocks shown in top view, continuing passage of the gas 2 hours. (At first a rather slow stream of gas passes in, but during last 15 min. flow is fairly rapid.) At end of reaction period discontinue flow of gas, and disconnect long tubes F, F', and F'' and the outlet tubes of three test tubes G, G', and G'' from O and P. (Tubes F, F', and F'' are pulled up just above surface of reaction mixture and are closed by means of short pieces of rubber tubing having one end plugged with short piece of glass rod.) Similarly close off outlet tube N and the outlet tubes of G' and G''. Place the tubes containing reaction mixture in cold room or icebox (temp.  $+8^{\circ}$ – $+10^{\circ}$ ) and allow to remain 24 hours. Transfer contents of tubes G, G', and G'' to 1 liter Erlenmeyer flasks, taking care to remove any material adhering either on inside or outside of tubes F, F', and F''. Dilute reaction mixtures with  $H_2O$  to volume of 500 ml. Connect flasks to reflux condensers and boil 1 hour. Prepare three Gooch crucibles in usual manner, dry at  $105^{\circ}$ , and weigh. Ignite one of weighed crucibles, A, on a Bunsen burner, cool in a desiccator, and reweigh. Allow contents of flasks to cool to room temp. and filter thru weighed Gooch crucibles. Wash precipitates collected in Gooch crucibles with hot  $H_2O$ , dry at  $105^{\circ}$ , and weigh in weighing bottle. Ignite the crude lignin in crucible A over Bunsen flame and determine weight of ash. Place one of the other two Gooch crucibles in Kjeldahl flask provided with wide neck, and determine percentage of N in the crude lignin as directed in II, 23. If it is desired to determine percentage of methoxyl in the lignin, collect precipitate from one of the flasks in a dried ( $105^{\circ}$ ) sintered glass crucible. Compute weight of lignin in sample as follows: Weight of lignin = weight of crude lignin – weight of ash – weight of crude protein ( $N \times 6.25$ ). Calculate percentage of lignin in the original dry unextracted material.

## SELECTED REFERENCES

- <sup>1</sup> Bot. Gaz., 73, 44 (1922); Proc. Am. Soc. Hort. Sci., 1927, p. 191; J. Assoc. Official Agr. Chem., 13, 224 (1930); 16, 71 (1933); 19, 70 (1936).
- <sup>2</sup> J. Assoc. Official Agr. Chem., 11, 203 (1928); 16, 70 (1933); 19, 70 (1936).
- <sup>3</sup> Ibid., 11, 207 (1928); 14, 72 (1931); 19, 70 (1936).
- <sup>4</sup> Ibid., 13, 221 (1930); 19, 359 (1936); J. Am. Chem. Soc., 51, 2730 (1929).
- <sup>5</sup> J. Biol. Chem., 7, 83 (1910); J. Assoc. Official Agr. Chem., 4, 392 (1921); 16, 70 (1933).
- <sup>6</sup> J. Biol. Chem., 47, 475 (1921); 50, 527, 537 (1922); J. Assoc. Official Agr. Chem., 14, 216 (1931); 16, 70 (1933); 19, 71 (1936).
- <sup>7</sup> J. Assoc. Official Agr. Chem., 3, 329 (1920); 4, 393 (1921); 16, 71 (1933); 19, 71 (1936).
- <sup>8</sup> Ibid., 4, 393 (1921).
- <sup>9</sup> Ibid., 19, 71 (1936).
- <sup>10</sup> Ibid., 12, 35 (1929).
- <sup>11</sup> U. S. Dept. Agr. Bur. Chem. Bull., 105, p. 151; 116, p. 92; 137, p. 30.
- <sup>12</sup> J. Assoc. Official Agr. Chem., 6, 415 (1923).
- <sup>13</sup> Ibid., 16, 71 (1933).
- <sup>14</sup> Ibid., 14, 216 (1931); J. Biol. Chem., 59, 255 (1929).
- <sup>15</sup> J. Assoc. Official Agr. Chem., 11, 209 (1928); 12, 195 (1929); 21, 107 (1938).
- <sup>16</sup> Sutton, Systematic Handbook of Volumetric Analysis, 16th Ed., 1911, p. 146; J. Am. Chem. Soc., 37, 1128 (1915).
- <sup>17</sup> J. Assoc. Official Agr. Chem., 18, 379 (1935); 19, 72 (1936).
- <sup>18</sup> Ibid., 18, 73 (1935).
- <sup>19</sup> Ibid., 19, 236 (1936).
- <sup>20</sup> Ibid., 14, 73, 225 (1931); 15, 71 (1932).
- <sup>21</sup> J. Am. Chem. Soc., 43, 1503 (1921); J. Assoc. Official Agr. Chem., 15, 71 (1932).
- <sup>22</sup> J. Assoc. Official Agr. Chem., 19, 72 (1936).
- <sup>23</sup> Ibid., 14, 229 (1931); 16, 71 (1933).
- <sup>24</sup> Ibid., 16, 474 (1933); 17, 63 (1934).
- <sup>25</sup> Ibid., 15, 124 (1932); 18, 386 (1935); 19, 107 (1936).

### XIII. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

#### 1 PRELIMINARY EXAMINATION<sup>1</sup>

Note and record (a) appearance, whether bright or turbid, or any sediment; (b) color and depth of color; (c) odor, whether fruity, foreign, or artificial; (d) taste, whether tart or sweet, fruity, artificial or foreign, and whether any synthetic substance can be identified by odor or taste.

#### 2 SPECIFIC GRAVITY—OFFICIAL.—See XIV, 3.

#### 3 ALCOHOL—OFFICIAL.—See XIV, 5.

#### 4 TOTAL SOLIDS—OFFICIAL.—See XXXIV, 4 or 5.

#### SUCROSE

#### 5 *By Polarization—Official*

Determine by polarizing before and after inversion as directed under XXXIV, 23 or 24.

#### 6 *By Reducing Sugars Before and After Inversion—Official.—See XXXIV, 29.*

#### 7 REDUCING SUGARS—OFFICIAL

Use the value for reducing sugars before inversion, 6.

#### 8 COMMERCIAL GLUCOSE—OFFICIAL.—See XXXIV, 31.

#### 9 ASH—OFFICIAL

Proceed as directed under XXXIV, 9 or 10, using a quantity of sample that contains not more than 10 g of solids.

#### 10 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using ash obtained under 9.

#### 11 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using soluble ash obtained under 10.

#### 12 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 15, using insoluble ash obtained under 10.

#### 13 ANALYSIS OF THE ASH—OFFICIAL.—See XII and XXVI.

#### 14 PRESERVATIVES AND ARTIFICIAL SWEETENERS—OFFICIAL.—See XXXII.

#### 15 COLORING MATTERS—TENTATIVE.—See XXI.

#### 16 METALS—TENTATIVE.—See XXIX.

#### 17 TOTAL ACIDITY—OFFICIAL.—See XVI, 55.

#### CHARACTERISTIC ACIDS<sup>2</sup>

#### 18 PRELIMINARY PROCEDURE

(a) *Alcoholic products.*—Proceed as directed under XVI, 56.

(b) *Non-alcoholic products.*—Measure out volume of sample that contains not more than 30 g of solid matter and not more than 200 mg of the acid to be deter-

mined as calculated from the acidity. Evaporate to 30 ml if necessary, add 3 ml of 1 N  $\text{H}_2\text{SO}_4$  (except in determining malic acid), and transfer to 250 ml volumetric flask, using 10 ml of  $\text{H}_2\text{O}$  and sufficient alcohol to fill flask to mark. Mix, and allow to stand 15 min. Filter thru thin layer of absorbent cotton, protecting liquid against evaporation. Transfer 200 ml of filtrate to centrifuge bottle and proceed as directed below.

19

## TARTARIC ACID—TENTATIVE

Using the material in the centrifuge bottle, proceed as directed under XXVI, 27 or 29.

20

## CITRIC ACID—TENTATIVE

Using the material in the centrifuge bottle, proceed as directed under XXVI, 31.

21

## MALIC ACID—TENTATIVE

Using the material in the centrifuge bottle, proceed as directed under XXVI, 34.

22

## VOLATILE ACIDS—OFFICIAL.—See XV, 23.

23

## ESTERS—OFFICIAL.—See XVI, 60.

## ANTHRANILIC ACID ESTER

*Colorimetric Method<sup>3</sup>—Official*

(Use when sample contains less than 500 mg per liter.)

24

## REAGENTS

- (a) *Hydrochloric acid soln.*—Dilute 83 ml of  $\text{HCl}$  to 1 liter with  $\text{H}_2\text{O}$ .
- (b) *Sodium nitrite soln.*—Dissolve 2 g of  $\text{NaNO}_2$  in 100 ml of  $\text{H}_2\text{O}$ .
- (c) *Hydrazine sulfate soln.*—Dissolve ca 3 g of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$  in 100 ml of  $\text{H}_2\text{O}$ .
- (d) *Sodium- $\alpha$ -naphthol-2-sulfonate soln.*—Dissolve 5 g of the sulfonate in 100 ml of  $\text{H}_2\text{O}$ .
- (e) *Sodium carbonate soln.*—Dissolve 25 g of  $\text{Na}_2\text{CO}_3$  in 75 ml of  $\text{H}_2\text{O}$ .
- (f) *Standard soln of methyl anthranilate.*—Dissolve 0.25 g of methyl anthranilate in 60 ml of alcohol and dilute with  $\text{H}_2\text{O}$  to 250 ml.

25

## APPARATUS

- (a) *Steam generator filled with  $\text{H}_2\text{O}$ .*—Oil can holding 1 gallon will serve purpose.
- (b) *Distillation flask.*—Kjeldahl flask of ca 750 ml capacity, with shortened neck, ca 10" in height over all.
- (c) *Spray tube.*—Glass tube with small perforated bulb at end. Passes thru rubber stopper and reaches to bottom of distillation flask.
- (d) *Connecting bulb.*—Kjeldahl bulb with bent connecting tube.
- (e) *Worm condenser.*—Having water jacket 10–12" long. Outlet tube is extended to reach bottom of receiving flask.
- (f) *Receiving flask.*—500 ml Erlenmeyer flask.

26

## DETERMINATION

Place enough  $\text{H}_2\text{O}$  in receiving flask to just cover or seal end of extended condenser tube. Place 10–100 ml of sample in distillation flask; add, if necessary, sufficient  $\text{H}_2\text{O}$  to make volume 100 ml; insert stopper carrying spray tube and connecting bulb; and connect with condenser and receiving flask. Immerse distilla-

tion flask in water bath to level of contents, and when sample has attained temp. of the nearly boiling bath connect with steam generator and pass rapid current of steam thru sample until ca 300 ml of distillate has been collected.

Disconnect apparatus and wash out condenser with a little  $\text{H}_2\text{O}$ . Add to distillate 25 ml of the  $\text{HCl}$  soln and 2 ml of the  $\text{NaNO}_2$  soln, mix well, and let stand exactly 2 min. Add 6 ml of the  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$  soln and mix well for a minute, so that liquid comes in contact with all parts of flask that may have been touched by the soln when it contained free nitrous acid. Keep liquid in flask in rapid motion, add quickly 5 ml of the  $\text{Na-}\alpha$ -naphthol-2-sulfonate soln, and then add immediately 15 ml of the  $\text{Na}_2\text{CO}_3$  soln. Dilute the colored soln to 500 ml with  $\text{H}_2\text{O}$ , mix, and compare color of aliquot with color of a standard, or set of standards, prepared as nearly as possible at same time. Calculate and express results as mg of anthranilic acid ester, as methyl anthranilate, per liter of sample.

*Gravimetric Method\*—Official*

(Use when sample contains 500 mg or more per liter.)

27

REAGENTS AND APPARATUS

(a)  $\alpha$ -naphthol soln.—Dissolve 0.2 g of  $\alpha$ -naphthol in 100 ml of 30% (by volume) alcohol.

(b) Sodium bicarbonate soln.—Dissolve 8.4 g of  $\text{NaHCO}_3$  in 100 ml of  $\text{H}_2\text{O}$ .

See 24 for other reagents and see 25 for apparatus.

28

DETERMINATION

Place in the distillation flask a quantity of the sample that contains 50–125 mg of anthranilic acid ester and dilute, if necessary, to 100 ml with  $\text{H}_2\text{O}$ . Subject sample to steam distillation as directed in 26, collecting ca 400 ml of distillate. Have the  $\text{H}_2\text{O}$  in bath near b.p. when bath is placed under distillation flask, also have the  $\text{H}_2\text{O}$  in steam generator boiling and make connection immediately.

Wash out condenser with a little  $\text{H}_2\text{O}$  and dilute distillate to 500 ml. Mix, and to 200 ml aliquot add 5 ml of the  $\text{HCl}$  soln and 5 ml of the  $\text{NaNO}_2$  soln. Mix well and let stand 1 min. Mix 25 ml of the  $\alpha$ -naphthol soln and 6 ml of the  $\text{NaHCO}_3$  soln, pour the diazotized soln into mixture, and let stand 10 min. Fold two Whatman No. 1 or S. & S. No. 595 filter papers, 12.5 cm in diameter, and determine difference in their weights by placing one on each pan of balance and counterpoising with added weights. Place the heavier inside the lighter paper, fit into funnel, and moisten. Pour mixture thru this filter and wash precipitate 7 or 8 times, using total of ca 100 ml of  $\text{H}_2\text{O}$ . Fill filter only to within 1 cm of top. Place funnel carrying filter and washed precipitate in oven, and dry ca 10 min. at  $100^\circ$ . Separate filter papers and dry them ca 1 hour at same temp. Ascertain difference in weights, dry again, weigh again, and repeat this procedure until difference in weights remains constant. From this constant difference in weights subtract original difference in weights of the 2 filter papers and multiply result by 0.4935 to obtain weight of anthranilic acid ester, as methyl anthranilate. Report as grams per liter of sample.

29

BENZALDEHYDE\*—TENTATIVE

Measure into distilling flask 500 ml of the beverage, 100 ml of flavoring sirup, or 10–25 ml of flavor; add 32 ml of alcohol, and in the case of sirup or flavor, ca 300 ml of  $\text{H}_2\text{O}$ , and proceed as directed in XVI, 64.

30

GAMMA UNDECALACTONE<sup>a</sup>—TENTATIVE

Proceed as directed under XVI, 61, using 500 ml of beverage, 100 ml of flavoring sirup, or 10–50 ml of flavor.

## SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., 19, 74 (1936).

<sup>2</sup> Ibid., 75.

<sup>3</sup> Ibid., 11, 46 (1928).

<sup>4</sup> Ibid., 47.

<sup>5</sup> Ibid., 19, 75 (1936).

<sup>6</sup> Ibid., 16, 420 (1933); 19, 75 (1936).



## XIV. MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

### BEER

(Unless otherwise directed express results as g per 100 g.)

#### 1 PREPARATION OF SAMPLE—OFFICIAL

Remove CO<sub>2</sub> by transferring sample to large flask and shaking gently at first and then vigorously, or by pouring back and forth between beakers, preferably brass, nickel, or copper (tin lined), and maintain temp. of beer at 20–25°. Eliminate foam by passing the CO<sub>2</sub>-free beer thru dry filter paper.

#### 2 COLOR—OFFICIAL, FIRST ACTION

Determine depth of color of sample in  $\frac{1}{2}$ " cell with Lovibond tintometer, using Series 52 slide. Express result in terms of  $\frac{1}{2}$ " cell. Use standard daylight lamp, 46. Filter beers showing opacity.

#### 3 SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° (in air) by means of pycnometer as follows: Carefully clean pycnometer by filling with saturated soln of CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub>, allowing to stand several hours, emptying, and rinsing thoroly with H<sub>2</sub>O. Fill pycnometer with recently boiled H<sub>2</sub>O previously cooled to 2–4° below desired temp., place in water bath cooled to same temp., and allow bath to warm slowly to desired temp. Adjust level of H<sub>2</sub>O to proper point on pycnometer; put perforated cap or stopper in place, remove pycnometer from bath, wipe dry with clean cloth, and after allowing to stand 15–20 min., weigh. Empty, rinse several times with alcohol, and then with ether, remove ether fumes, allow pycnometer to become perfectly dry, and weigh. Ascertain weight of contained H<sub>2</sub>O at desired temp. in air (W of formula below) by subtracting weight of empty pycnometer from its weight when full. Cool sample to 2–4° below desired temp., fill pycnometer, adjust level of liquid to proper point on pycnometer at desired temp., put perforated cap or stopper in place, wipe dry, and weigh as before. Ascertain weight of contained sample at desired temp. in air (S of formula below) by subtracting weight of empty pycnometer from its weight when filled with sample. Sp. gr. = weight of contained beer ÷ weight of contained H<sub>2</sub>O. Use the Plato table, 3, XLIII, to ascertain apparent extract or saccharometer indication. Calculate sp. gr. in vacuo, if desired, by following formula:

$$G = \frac{S + 0.00105W}{W + 0.00105W}, \text{ in which}$$

$G$  = corrected sp. gr. of sample at desired temp. in vacuo;

$W$  = weight of contained H<sub>2</sub>O at desired temp. in air; and

$S$  = weight of contained sample at desired temp. in air.

#### 4 APPARENT EXTRACT OR SACCHAROMETRIC INDICATION<sup>1</sup>—OFFICIAL, FIRST ACTION

From the Plato table, 3, XLIII, ascertain apparent extract corresponding to sp. gr. determined at 20/20° and report to second decimal place.

#### 5 ALCOHOL—OFFICIAL

(a) *By volume*.—Measure 100 ml of the liquid into 300–500 ml distillation flask, noting temp., and add 50 ml of H<sub>2</sub>O. Attach flask to vertical condenser by means

of bent tube, distil almost 100 ml, and make to volume of 100 ml at same temp. Determine sp. gr. of distillate as directed under 3, at room temp. if desired, and obtain corresponding percentage of alcohol by volume from Table 19, XLIII.

(b) *By weight*.—From Table 21, XLIII, obtain % alcohol by weight in the distillate corresponding to % alcohol by volume, multiply by the sp. gr. of distillate, and divide by sp. gr. of the sample.

(c) *By immersion refractometer*.—Verify percentages of alcohol, as determined under (a) and (b), by ascertaining immersion refractometer reading of distillate and obtaining corresponding percentages of alcohol from Table 20, XLIII.

6

## REAL EXTRACT—OFFICIAL

(a) Calculate sp. gr. of the dealcoholized beer by following formula:

$S = G + 1 - A$ , in which

$S$  = the sp. gr. of dealcoholized beer;

$G$  = the sp. gr. of beer; and

$A$  = the sp. gr. of distillate obtained in determination of alcohol, 5(a).

From Table 3, XLIII, ascertain percentage by weight of extract in dealcoholized beer corresponding to value of  $S$ . The figure thus obtained  $\times S$  = grams of extract per 100 ml of beer.

(b) Evaporate on water bath or asbestos plate 75–100 ml of sample (accurately weighed to within 0.1 g) to ca  $\frac{1}{3}$  of its original volume, but do not allow temp. to exceed 80°. Cool, make up to original weight with  $H_2O$  and determine sp. gr. with pycnometer at 20/20°. (If too much  $H_2O$  has been added, the sp. gr. will be proportionately too low, and a correction must be made.) Ascertain real extract directly from the Plato table, 3, XLIII.

(c) If no anti-foam material was used in the determination of alcohol, 5, transfer residue quantitatively with hot  $H_2O$  to 100 ml flask. Cool, and make up to 100 ml at 20°. Determine sp. gr. at 20/20°, 3, and ascertain the extract direct from the Plato table, 3, XLIII. If 100 ml of beer was taken, make following correction:

$$\text{Extract found} \times \frac{\text{sp. gr. of dealcoholized beer}}{\text{sp. gr. of beer}} = \text{g of extract in 100 g of beer.}$$

7

## REAL EXTRACT, OFFICIAL, FIRST ACTION

Immersion refractometer reading of beer at 20° minus refractometer reading of distillate at 20°  $\times 0.2571$  = grams of extract in 100 ml of beer.

8

## EXTRACT OF ORIGINAL WORT—TENTATIVE

Calculate from following formula and report to first decimal place.

$$O = \frac{(A \times 2.0665) + E}{100 + (A \times 1.0665)} \times 100, \text{ in which}$$

$O$  = extract of original wort;

$A$  = % alcohol by weight (g per 100 g of beer); and

$E$  = real extract, 6(b) or 6(c).

9

## REAL DEGREE OF FERMENTATION OR REAL ATTENUATION—TENTATIVE

Calculate as follows and report to first decimal place.

$$\frac{\text{orig. ext.} - \text{real ext.}}{\text{orig. ext.}} \times 100.$$

10

## TOTAL ACIDITY—TENTATIVE

Run 10 or 25 ml of decarbonated beer, 1, into ca 10 volumes of boiling  $H_2O$ , and continue boiling 60 seconds or longer after addition of sample. Cool to room temp. and titrate immediately with 0.1 *N* alkali, using 0.5 ml of 0.5% phenolphthalein. 1 ml of 0.1 *N* alkali = 0.0090 g of lactic acid. Report results to nearest 0.01%.

11

## VOLATILE ACIDS—OFFICIAL

Using 100 ml of beer, proceed as directed under XV, 24. Express result as acetic acid, g per 100 ml. 1 ml of 0.1 *N* alkali = 0.006 g of acetic acid.

12

## REDUCING SUGARS—OFFICIAL

Dilute 25 ml of prepared sample, 1, measured at 20°, to 100 ml with  $H_2O$  at same temp. Determine reducing sugars in 25 ml of this soln as directed under XXXIV, 32 and 37. Express result as grams of maltose per 100 ml of beer.

13

## DEXTRIN—TENTATIVE

To 50 ml of prepared sample, 1, measured at 20° in Erlenmeyer flask, add 15 ml of HCl (sp. gr. 1.125) and dilute to 200 ml. Attach flask to reflux condenser, and keep in boiling water bath 2 hours. Cool, nearly neutralize with NaOH, make up to volume of 250 ml, filter, and determine dextrose as directed under XXXIV, 48 or 49. From number of g of dextrose per 100 ml of beer, subtract 1.053 times quantity of maltose, 12, and multiply remainder by 0.9. The result is number of g of dextrin per 100 ml of beer.

14

## DIRECT POLARIZATION—TENTATIVE

Read polarization of original sample in °Ventzke in 200 mm tube at 20°. If beer is turbid, clarify by shaking with alumina cream, filter, and correct reading for dilution.

15

## GLYCEROL—OFFICIAL.—See XV, 6.

16

## ASH—OFFICIAL

Evaporate to dryness 50 ml of prepared sample, 1, measured at 20°, and proceed as directed under XXXIV, 9 or 10.

17

## PHOSPHORIC ACID—OFFICIAL

To 50 ml of prepared sample, 1, measured at 20°, add 20 ml of 2% Ca acetate soln, evaporate to dryness, and ignite at low redness to white ash. Add 10–15 ml of boiling  $HNO_3$  (1+9) and determine  $P_2O_5$  as directed under II, 12.

18

## PROTEIN—OFFICIAL

To 25 ml of prepared sample, 1, at 20° in Kjeldahl digestion flask, add 2–3 ml of  $H_2SO_4$  (1+1), and concentrate to sirupy consistency. Determine N as directed under II, 21, 22 or 23. Multiply result by 6.25 to calculate percentage of protein.

CARBON DIOXIDE IN BEER<sup>2</sup>—TENTATIVE*Manometric Method*

19

## APPARATUS

*Piercing apparatus.*—A gas-tight packing box and fastening for adjustment over crown of bottle, which holds a hollow spike. (A suitable apparatus may be obtained

from a number of manufacturers.) With a can a metal frame, the top of which is pressed or screwed down and locked over can top, holds a hollow spike surrounded by compressible rubber sealing plug. The spike leads to accurate pressure gage and outlet valve. (One apparatus, adjustable for bottles and cans, may be used.)

*Absorption buret.*—The buret (Fig. 19) consists of graduated tube (0–6 ml graduated in 0.1 ml divisions and 6–25 ml graduated in 0.2 ml divisions), having bulb,

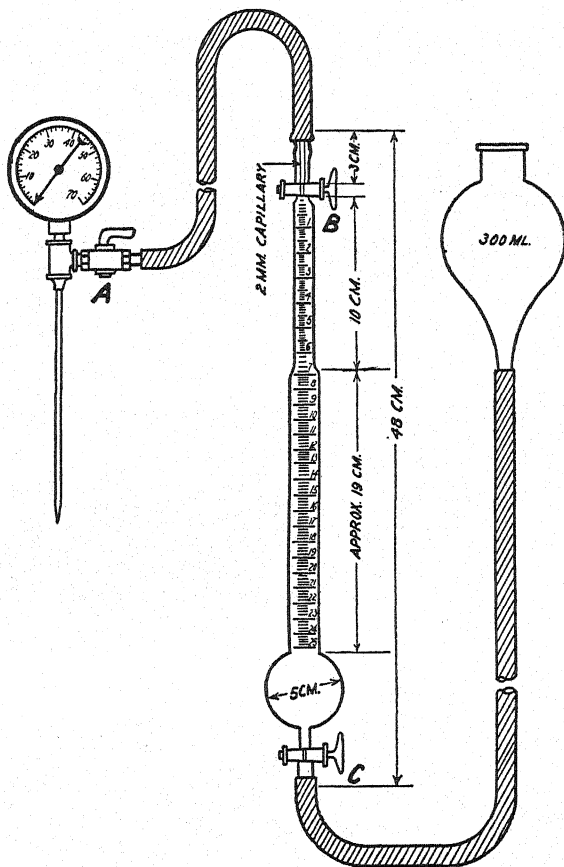


FIG. 19.—ABSORPTION BURET

and closed at each end by stopcocks. Upper end is connected by rubber tubing to outlet valve of piercing apparatus and lower end is connected by length of rubber tubing to leveling bulb.

If sample is in a bottle, make scratch mark at beer level; if sample is in a can, weigh can with contents. Submerge container in water bath at 25° long enough to bring temp. of beer to 25°. Connect piercing apparatus to the bottle or can. Fill absorption buret with 15% NaOH soln and allow soln to run up to stopcock B. Fill upper capillary of absorption bulb with hexyl alcohol and remainder of the sys-

tem between B and the tip of the spike with  $\text{H}_2\text{O}$  in order to displace any air. With outlet valve A closed, drive spike through crown or can top and thoroly shake and tap the bottle or can. Make pressure reading on gage. Again shake and take pressure readings. Use the pressure reading that shows no change in consecutive readings.

Open stopcocks B and C of absorption buret and then outlet valve A. Allow the gas, together with foam, to flow over into absorption buret. Swirl contents of buret to permit absorption of  $\text{CO}_2$ . When  $\frac{1}{2}$ – $\frac{3}{4}$  of the alkali soln in absorption buret has been displaced, shut off all stopcocks and shake to permit complete absorption of  $\text{CO}_2$ . Set buret in vertical position, open bottom stopcock, C, and allow alkali to flow back into bulb. Open stopcocks B and A and repeat above operation, tapping bottle or can to accelerate evolution of  $\text{CO}_2$ . Close upper stopcocks A and B and shake thoroly to absorb last traces of  $\text{CO}_2$ . Bring leveling bulb to such a position that height of soln in leveling bulb and buret is the same and read unabsorbed gas, which is reported as "air." Repeat operations until consecutive readings as to "air" are the same.

Disconnect bottle or can and determine head-space volume as follows:

If sample is a bottle, fill with  $\text{H}_2\text{O}$  to top and pour off into graduated cylinder to scratch mark. Number of ml of  $\text{H}_2\text{O}$  thus poured off represents head space in ml. If sample is a can, weigh empty can after pouring out all remaining beer. Difference represents weight of beer, which divided by sp. gr. of beer will give volume of beer in ml. Fill empty can with  $\text{H}_2\text{O}$  and weigh. Weight of  $\text{H}_2\text{O}$  in grams is also the volume in ml, so that difference between volume of  $\text{H}_2\text{O}$  and volume of beer represents head space in ml.

Calculate  $\text{CO}_2$  by weight by following formula:

$$\% \text{CO}_2 = \left[ P - \left( \frac{\text{ml of "air"}}{\text{ml of head space}} \times 14.7 \right) \right] \times 0.00965, \text{ in which}$$

P = absolute pressure in pounds per sq. in. at  $25^\circ$  = (ordinary gage pressure + 14.7)  
(For routine work 15 may conveniently be substituted for 14.7. Pounds per sq. in.  $\times 0.070307$  = kg per sq. cm.)

## 21

## SULFUR DIOXIDE—OFFICIAL, FIRST ACTION

Proceed as directed under XXXII, 32, except to add thru the dropping funnel 300 ml of the beverage (not decarbonated), with no additional  $\text{H}_2\text{O}$ , followed by 20 ml of  $\text{HCl}$ . Allow mixture to stand a few minutes until fumes have settled. Adjust burner so that the vapors rise no higher than one-tenth the length of water jacket of condenser and boil sample 90 min. Adjust flow of  $\text{CO}_2$  so that a slow, but steady stream passes thru receiver during the distillation and complete the analysis as directed in XXXII, 32. Report results as mg of  $\text{SO}_2$  per liter.

## 22

## IODINE REACTION FOR UNCONVERTED STARCH

(a) *Official, first action.*—(1) Place 10 ml of beer in one test tube, (2) in a second test tube place 0.1 N I soln (dissolve 12.69 g of I and 25 g of KI in  $\text{H}_2\text{O}$  and make up to 1 liter) and dilute to same color as beer. Slowly pour the I soln into the beer and note color. A normal beer should not change in color. A blue color indicates starch; a purple color, amyloextrine; and a reddish color, erythroextrine. No change in color indicates complete conversion.

(b) *For dark beer, but applicable also to light beer—Tentative.*—To 5 ml of beer in test tube add 25 ml of alcohol. Shake thoroly. Let stand. Decant, pouring off last trace of the beer-alcohol mixture. Dissolve precipitate (dextrine) in 5 ml of  $\text{H}_2\text{O}$  and to this soln add dropwise a 0.1 N I soln diluted 5 times. See (a) for reaction.

23 COLORING MATTERS—TENTATIVE.—*See* XXI.24 METALS—TENTATIVE.—*See* XXIX.25 PRESERVATIVES AND SWEETENERS—OFFICIAL.—*See* XXXII.26 CHLORIDES IN BEER<sup>1</sup>—TENTATIVE

Place 100 ml sample in a Pt dish, add 20 ml of a 5% soln of  $\text{Na}_2\text{CO}_3$ , and proceed as directed under XII, 35, 37.

## 27 METHYL ALCOHOL (QUANTITATIVE)—TENTATIVE

Transfer to distilling flask a quantity of sample that contains 20–25 ml of absolute alcohol and distil slowly, collecting distillate in 50 ml volumetric flask. When nearly to mark, disconnect receiver and adjust to mark at room temp. with  $\text{H}_2\text{O}$ . Determine methyl alcohol as directed under XVI, 26. *See also* XVI, 25 and 31.

## 28 H-ION CONCENTRATION—TENTATIVE

Decarbonate sample, 1, and determine pH electrometrically. Report results to nearest 0.05 unit.

## 29 END FERMENTATION AS INCREASE IN DEGREE OF FERMENTATION—TENTATIVE

Ferment 250 ml of beer with active compressed brewers' yeast at 15–25° for 48 hours, providing the fermentation flask with  $\text{H}_2\text{O}$ , acid, or Hg seal. Filter, determine real extract, 6 or 7, and calculate real degree of fermentation, 9.

## MALT\*—TENTATIVE

## 30 SAMPLING

For complete descriptions of the trier, divider, sampler, and bushel weight tester, see Handbook of Official Grain Standards of the United States Department of Agriculture, 1934.

(a) *Bulk malt in cars or bins.*—Using 60" trier, take at least 6 probes from different parts of car, preferably 2 from center and 2 from each end.

(b) *Bulk malt during discharge through spouts or openings.*—At different times during filling or unloading of a car take, with the trier or sampler, at least 6 samples, each representing a complete cross section from the grain stream coming out of spout.

(c) *Bagged malt.*—Sample not less than 2% of bags, lengthwise thru center of the open bags, using short trier.

Indicate approximate proportion of inferior grain and take representative samples from each portion in manner outlined above. Immediately place each portion of sample in suitable large dry container and keep tightly closed.

## 31 PREPARATION OF SAMPLE

Divide samples either by quartering or by using sample divider, until ca 1 lb. remains. Place reduced sample in air-tight container (preferably tin with screw or friction type cover). Do not use cartons, bags, wooden boxes, glass Mason jars, or wrapping paper for this purpose. Remove foreign particles, such as stone, wood, and twine. Do not remove foreign seeds or dust particles.

## 32 BUSHEL WEIGHT

Place sample in filling hopper of Winchester tester, open slide underneath, and

\* These methods are the official methods for analysis of malt adopted by the American Society of Brewing Chemists, October, 1935, edited to conform in part to the style of this publication.

allow malt to fill measuring cylinder to overflowing. Without jarring, level off with straight-edge longer than diameter of measuring cylinder, making one forward stroke consisting of three distinct zig-zag motions. Weigh to nearest  $\frac{1}{4}$  pound.

33

## LENGTH OF ACROSPIRE

Quarter sample until ca 200 kernels remain in two opposite quarters. Count out 100 kernels, remove husk covering the acrospire by means of sharp instrument (knife or tweezer with sharpened edge), and note acrospire length in comparison with length of kernel. Divide kernels into following classifications: 0 to  $\frac{1}{4}$ ,  $\frac{1}{4}$  to  $\frac{1}{2}$ ,  $\frac{1}{2}$  to  $\frac{3}{4}$ ,  $\frac{3}{4}$  to 1, overgrown. Include  $\frac{1}{4}$ ,  $\frac{1}{2}$  and  $\frac{3}{4}$  grown kernels in upper classification. Count number of kernels belonging to each class.

34

## MEALINESS

Count out 100 kernels remaining from preceding test and cut them in longitudinal halves. Determine percentage of mealy, half glassy, and glassy kernels. In case of uncertainty, pierce starch body with sharp point; if mealy, it will break away and crumble from the point.

35

## 1,000 KERNEL WEIGHT

Quarter sample until ca 500 kernels remain in two opposite quarters. Count out 500 kernels and weigh to nearest 0.1 g. Calculate results to 1,000 kernels on "as is" and dry basis.

36

## ASSORTMENT\*

Weigh 100 g from a quartered sample to nearest 0.1 g. Place in top compartment of grader and shake 3 min. Weigh quantities remaining on various screens and in catch pan to nearest 0.1 g, and report percentage on each of following screens: 8/64", 7/64", 6 $\frac{1}{2}$ /64", 5 $\frac{1}{2}$ /64", 5/64", and thru the 5/64", in percentage totaling 100%.

37

## MOLD

Determine presence or absence of mold by visual inspection and report as "none," "trace," etc.

38

## FOREIGN SEEDS AND BROKEN KERNELS

Weigh 50 g of sample. Pick out foreign seeds and broken kernels, classify, and report separately in percentage.

## MOISTURE—OFFICIAL, FIRST ACTION

39

## APPARATUS

(a) *Weighing dish*.—Use glass bottle, or an Al dish, provided with tight fitting cover and having diameter of ca 40 mm for a 5 g sample, or 55 mm for 10 g sample.

(b) *Oven*.—Should have an automatic temp. control capable of holding temp. within  $\pm 0.5^\circ$  C. and be large enough to accommodate all the samples on one shelf in such a manner that no sample is outside area indicated by test to give comparable results in duplicate samples. Standardize oven as follows: Place weighed duplicate samples in oven at 103–104° and dry 3 hours. Weigh, and redry 1 hour longer. If loss of moisture is more than 0.1%, raise temp. 1° and again test with new duplicate samples. Take as standard the lowest temp. below 106° giving moisture content which, after 3 hours of drying, is within 0.1% of value attainable at same temp. within 4 hours. Keep ventilators of oven open during entire drying period, and do not open door during the 3 hours of drying.

\* Grader manufactured by the Richmond Mfg. Co., Lockport, N. Y.

40

## PREPARATION OF SAMPLE

(a) *If extract determination is to be made.*—Grind as instructed under "Extract," and transfer in one continuous operation. When many samples are used, grind first sample, remove beaker, and grind second sample while adjusting weight of first sample. Remove second sample, insert third sample, and repeat operation.

(b) *If extract determination is not made.*—Have sample of same fineness as the finely ground malt used for determination of extract. Weigh ca 5 g of whole malt and grind thru clean dry mill directly into weighing bottle (10 g may be taken provided the 55 mm diameter weighing bottle is used). Brush all malt from mill into weighing bottle and cover immediately.

41

## DETERMINATION

Weigh sample to 0.1 mg and place in oven previously heated to standard temp. Remove cover of weighing bottle and heat exactly 3 hours at the standard temp. Transfer weighing bottle, with cover replaced, to desiccator and when cooled to room temp. weigh to 0.1 mg. Report moisture to nearest 0.1 %.

## EXTRACT—OFFICIAL, FIRST ACTION

42

## REAGENT

*Iodine soln.*—0.01 N. Dissolve 0.63 g of I and 1.25 g of KI in H<sub>2</sub>O and make up to 500 ml. Prepare fresh soln every month.

43

## APPARATUS

(a) *Mill.*—Miag-Seck. For fine grinding use the cone-type, 300 r.p.m., and for coarse grinding the roller-type, 150 r.p.m.

(b) *Sieves.*—Set of six 8", half-height U. S. standard sieves Nos. 10, 14, 18, 30, 60, and 100 (with pan and cover).

(c) *Mash beakers and counter weights.*—Made of either pure nickel or brass, not copper, and of such dimensions as to assure tight connection between beakers and the Miag-Seck mill during grinding period.

If counter weights are used for the mash beakers, check their tare weight frequently.

(d) *Mashing apparatus.*—Beakers, stirrers, and solder shall be made of the same metal. Each stirrer shall be provided with a blade, which in operation has clearance of ca 2 mm from bottom and 5 mm from wall of mash beaker. The blade shall be ca 8 mm wide, and each side shall have a pitch of 45°, arranged as in a propeller, to cause an upward motion of mash. The speed of mash stirrer shall be 80–100 r.p.m., each stirrer of each beaker having the same speed. Stir the H<sub>2</sub>O in the bath thoroly by mechanical means to assure uniformity of temp. and have the level of the H<sub>2</sub>O above the maximum mash level.

(e) *Gypsum plate.*—Prepare by thoroly mixing 100 ml of H<sub>2</sub>O with 135 g of plaster of Paris. Pour this mixture, while still free-flowing, into suitable flat molds (cigar boxes, etc.).

(f) *Filter paper.*—Use Schleicher and Schüll 32 cm fluted filter paper No. 560 (or No. 597, 32 cm to be fluted by analyst) or Delta Düren, 32 cm fluted filter paper No. 314½ (or No. 314, 32 cm, to be fluted by analyst).

(g) *Funnels.*—Use short-stemmed glass funnels of ca 20 cm diameter and do not allow filter paper to project above rim of funnel. Stem shall extend ca 3–5 cm into receiving flask.

(h) *Flasks.*—Use dry 500 ml Erlenmeyer flasks. Mark at 100 ml level.



(i) *Pycnometers*.—Any suitable pycnometer but preferably of Reischauer type, which is approximately 15 cm high and has a neck ca 9 cm long and an internal neck diameter between 2.5 and 3.5 mm. Place thin, well defined mark 55–70 mm below upper rim of neck. When filled with  $H_2O$  at  $20^\circ$  it shall have capacity of 48–50 g. Use glass funnels having capacity of ca 15 ml to fill the pycnometers.

(j) *Emptying device for Reischauer pycnometer*.—Bend piece of non-ferrous metal capillary tubing (brass, stainless steel) of less than 2 mm outside diameter to angle of ca  $45^\circ$ . The end to be inserted into pycnometer shall reach within few mm of bottom. Connect other end either to rubber aspirator bulb or to compressed air supply not exceeding 5 lbs. per sq. in.

(k) *Water bath*.—Use an automatically controlled water bath. If one is not available, use following set-up: Have water level of bath (5–15 liters capacity) reach above neck marks of pycnometer, keep water bath temp. at  $20^\circ (\pm 0.05^\circ)$ , and read on accurate thermometer, calibrated to  $1/10^\circ$ . Maintain temp. of water bath by very slow but continuous flow of ice water from container (2–4 liters capacity, containing ice and  $H_2O$ ). Regulate flow of ice water by hand. Stir the  $H_2O$  in bath mechanically and continuously without splashing.

44

## STANDARDIZATION

(a) *Setting of mill*.—Use malt of the following characteristics:

Moisture .....	4.0–5.0%
Extract in finely ground malt (Plato) as is.....	68–71%
Color of laboratory wort, Lovibond series 52- $\frac{1}{2}$ " cell. ...	1.5–2.2°
1000 kernel weight, as is .....	22–26 g

Acrospire development: 80% of kernels to have an acrospire longer than  $\frac{1}{2}$  of kernel length; not more than 5% of kernels to have acrospires longer than kernel length.

*Fine grinding*.—Weigh ca 51 g of the specified malt into mash beaker, grind, and collect in same beaker. Sieve 50 g of the well-mixed ground malt thru standard sieves. Shake sieves with malt by hand in horizontal plane 5 min., tapping set of sieves upon table top every 15 seconds. Detach top sieve from other sieves and shake for short time over sheet of paper until no particles are emitted. Add particles to next sieve. Repeat procedure with sieves Nos. 14, 18, and 30. The mill shall be considered as having standardized setting when sum of ground malt portions remaining on sieves Nos. 10, 14, 18, and 30 is between 4.5 and 5.5 g (9–11%). Standardize mill at least twice yearly.

*Coarse grinding*.—Proceed as directed for *fine grinding*. The mill shall be considered as having standardized setting when sum of ground malt portions remaining on sieves Nos. 10, 14, 18, and 30 is between 29 and 31 g (58–62%).

(b) *Pycnometer*.—Clean interior and exterior of pycnometer with  $Na_2Cr_2O_7$ - $H_2SO_4$  soln, discharge carefully with air, and wash several times with  $H_2O$ , then with alcohol and finally with ether. To remove last traces of ether vapor and replace it with laboratory air, connect metal capillary tubing (dry) to vacuum and insert into pycnometer 1–2 min. Carefully wipe pycnometer, allow to stand a few minutes, and determine tare by weighing to 0.2 mg.

Fill with freshly distilled  $H_2O$  and place in water bath held at  $20^\circ (\pm 0.05^\circ)$ . Force out air bubbles by gentle tapping. After 25 min., remove liquid above mark by means of capillary pipet provided with small rubber bulb. Make final adjustment of meniscus by absorbing last quantity of liquid by means of thin strips of blotting paper; also remove any liquid adhering to inner surface of neck. Adjust water level

so that lower part of meniscus rests on mark. Make all adjustments of liquid level within pycnometer neck while holding it at the neck, but without touching body of pycnometer with the hands. Keep body of pycnometer submerged during entire period of meniscus adjustment.

Raise pycnometer to room temp. by insertion into water bath kept at exactly that temp., and hold 10 min. Remove pycnometer, carefully dry exterior, and weigh to 0.2 mg. The difference between the two weighings represents  $H_2O$  capacity of the pycnometer at 20°. Redetermine tare weight and  $H_2O$  capacity at least twice a year.

45

## DETERMINATION

*Fine grinding.*—Weigh ca 55 g of sample (at room temp.) into tared mash beaker and grind thru mill set for standardized fineness of grind. Collect finely ground malt in same mash beaker, carefully brushing malt particles remaining in mill into mash beaker. Without delay, place mash beaker with contents on balance (accurate to within  $\pm 0.05$  g under 750 g load) and adjust weight of malt to 50 g ( $\pm 0.05$  g) by removing excess into tared dish for determination of moisture.

*Coarse grinding.*—Weigh 50.5 g or less of sample (at room temp.) into tared mash beaker and grind thru the mill, set for standardized coarseness of grind. Collect coarsely ground malt in same mash beaker, carefully brushing particles remaining in mill into mash beaker. Without delay, place mash beaker with contents on balance (accurate to within  $\pm 0.05$  g under 750 g load) and adjust weight of malt to 50 g ( $\pm 0.05$  g) by removing excess.

(a) *Mashing procedure.*—"Mash in" ground malt with 200 ml of  $H_2O$  at 46° and mix well with glass rod to prevent formation of lumps. Carefully rinse glass rod and wall of beaker with small quantity of  $H_2O$ . Note odor of mash and report as aromatic, slightly aromatic, musty, green, stale, etc. Promptly place mash beakers in mashing apparatus containing  $H_2O$  previously heated to 46°, and set stirrers in motion. Place thermometer in each mash beaker. Maintain temp. of 45° exactly 30 min. from time beakers were placed in mashing apparatus. Raise mash temp. 1° per min. until 70° is reached. Add 100 ml of  $H_2O$ , previously heated to 70–71°, and hold mash at 70° 60 min. (Temp. deviations during mashing procedure should not exceed 0.5°.)

(b) *Conversion.*—Transfer a drop of mash by means of thin glass rod (ca 3 mm diameter) onto absorbent gypsum plate, and test with drop of the I soln, 42. Make tests 5, 7 and 10 min. after 70° has been reached, and thereafter, if necessary, at 5 min. intervals. Conversion is complete when test drop and I produce only yellow stain on gypsum plate. Report time of conversion in periods: less than 5 min., 5–7 min., etc. Time of conversion is not determined on coarsely ground malt.

(c) *Cooling and filtration.*—Cool mash promptly within 10–15 min. to prevailing room temp. Stop stirrers. Remove thermometers after mash particles adhering have been rinsed into beaker with  $H_2O$ . Remove each beaker with its stirrer from mashing apparatus. Rinse mash particles adhering to stirrer into beaker with  $H_2O$ . Dry outside of each beaker, taking care to remove moisture adhering to rim. Without delay, adjust weight of contents of mash beaker to 450.0 g ( $\pm 0.05$  g) by addition of  $H_2O$ .

Stir mash thoroly with glass rod, once when removing beakers from balance pan, and again immediately before pouring mash onto filter. The two stirrings shall be not less than 5 min., nor more than 15 min. apart. During stirring of cooled mash, take care to prevent splashing or spilling. Mix drops adhering to beaker wall into mash by rotary stirring with glass rod.

Pour entire contents of beaker into funnel provided with the specified filter paper.

Cover funnel with large watch-glass (ca 20 cm diameter) during entire filtration. Return first 100 ml of filtrate to filter. When no more liquid is present above filter cake, discontinue filtration and remove receiving flask containing wort for subsequent observations and tests. In the case of slow running worts, stop filtration after 2 hours. When filtration is complete, mix wort in receiving flask thoroly by rotary motion. Speed of filtration is normal if filtration is complete (as defined above) within 1 hour after returning the 100 ml filtrate to filter bed; slow, if filtration requires longer time. Observe degree of clarity and report as: clear, slightly hazy, or hazy.

Remove ca 100 ml of the wort for determination of color. The color is not determined on the wort from coarsely ground malt.

(d) *Specific gravity*.—Rinse empty pycnometer twice with ca 10 ml of wort, and if Reischauer pycnometer is used remove rinsings each time by means of emptying device. Fill with wort, place in water bath, and follow procedure under 44(b). Weigh filled pycnometer within 3 hours of completed filtration. The difference between this weight and that of empty pycnometer represents wort capacity of pycnometer at 20°. Calculate sp. gr. of wort to fifth decimal place, rounding off to 0.00005 or 0.00010, by dividing weight of wort by weight of the H<sub>2</sub>O.

No calculation is made of sp. gr. in vacuo. If duplicate determinations made by same analyst in different beakers differ by more than two units in fourth decimal place, repeat entire determination.

(e) *Extract*.—Ascertain extract yield of the wort by reference to the sp. gr. values given in Table 3, XLIII, and calculate extract yield of the malt by following formula:

$$\text{Extract "as is"} = \frac{P(800 + M)}{100 - P}, \text{ in which}$$

$P$  = extract in 100 g of wort (Plato, Table 3); and

$M$  = % H<sub>2</sub>O in the malt.

$$\text{Extract "dry basis"} = \frac{E \times 100}{100 - M}, \text{ where}$$

$E$  = extract "as is"; and

$M$  = % H<sub>2</sub>O in the malt.

Report extract "as is" or on "dry basis" only to first decimal place.

46

#### COLOR OF WORT—OFFICIAL, FIRST ACTION

Use Lovibond tintometer,  $\frac{1}{2}$ " cell, Series 52, brewers' type, and standard daylight lamp (A.S.T.M., D 218-34T, 1933, or its spectrophotometric equivalent). Place tintometer in a box shield of metal or wood, finished in dull black so as to prevent interference from reflected light. Mount in horizontal position directly in front of artificial daylight lamp. Substitute flashed opal glass for the milk glass usually provided with instrument. Have distance between opal glass and daylight lamp such as to project diffused light with absence of glare or shadow upon opal glass and have near surface of daylight filter 6" from opal glass.

Pour wort into cell as quickly as possible after filtration and match against the standard glasses. Subdivide down to  $\frac{1}{8}^\circ$  color glasses and report results to nearest tenth. If difficulty is experienced in reading color, filter that portion of wort to be used for color determination separately thru dry filter paper without filter-aid.

#### DIASTATIC POWER

Wash all glassware with acid cleaning soln, 44(b), then rinse with ordinary tap H<sub>2</sub>O not less than 4 times, and finally rinse with distilled H<sub>2</sub>O at least twice. Thoroly dry digestion flasks.

(a) *Acetate buffer soln.*—Dissolve 68 g of Na acetate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ) in 500 ml of normal acetic acid and make up to 1 liter with  $\text{H}_2\text{O}$ .

(b) *Fehling's soln.*—Standardize as directed in XXXIV, 32, 33. Check soln from time to time by estimating its oxidizing value against a standard soln of invert sugar according to customary analytical procedure.

(c) *Starch soln.*—Have final concentration represent 2 g of soluble starch (weighed on dry basis) in 100 ml of soln. (Use starch of such quality and grade that its solubility will be at least 1:50 in hot  $\text{H}_2\text{O}$ , that it will contain no dextrans, contain less than 0.5% reducing substances calculated as maltose, and have moisture content of 10–12%. A freshly made 2% soln shall have a pH of 4.5–4.7 without adjustment by use of a buffer. Subsequent batches of starch shall, when tested on a malt of ca 100° Lintner (dry basis) having other characteristics as specified under determination of extract in malt, show a variation no greater than  $\pm 3^\circ$  Lintner from value obtained by using original starch in parallel determination. Further additional batches of starch when purchased shall be tested in parallel with starch in use. No variation greater than  $\pm 3^\circ$  Lintner shall be permitted. (In no case shall a cumulative correction as referred to original starch approved above amount to more than  $5^\circ$  Lintner.) Macerate starch with only sufficient cold freshly distilled  $\text{H}_2\text{O}$  to form a smooth thin paste (not over 5% of final volume). Pour this, with constant stirring, into boiling freshly distilled  $\text{H}_2\text{O}$  representing not less than ca 75% of final volume of the starch soln, at such rate that boiling does not cease. Continue boiling 2 min. after thin paste is completely introduced. Quickly add to beaker an additional 10% of the final volume of cold freshly distilled  $\text{H}_2\text{O}$  and transfer mixture quantitatively to glass-stoppered volumetric flask, mix by inverting flask, wash down neck on flask, and cool to  $20^\circ$  before adding buffer soln. Add 2 ml of the buffer soln for each 100 ml of final volume of starch soln and make up to mark. Mix again by inverting flask and keep tightly stoppered at  $20^\circ$  until used.

Grind separately not over 25.5 g of malt as directed under 45. Collect the finely ground malt in mash beaker, carefully brushing in the malt particles remaining in mill. Without delay, adjust weight of contents to 25 g ( $\pm 0.05$  g). Transfer quantitatively to container (capacity ca 1 liter) in which infusion is to be made. Add 500 ml of freshly distilled  $\text{H}_2\text{O}$  and close container. Let infusion stand 2.5 hours at  $20^\circ$  ( $\pm 0.2^\circ$ ) and agitate by rotation at 20 min. intervals. Take care that in agitation of malt suspension as small a quantity as possible of grist is left adhering to inner surface of flask above level of the  $\text{H}_2\text{O}$ . (Mixing by inverting the flask is specifically cautioned against. Gentle whirling of contents without splashing on sides of container has been found to give sufficient mixing.) Filter infusion by transferring entire charge to 30–32 cm fluted filter (CS and S No. 588) contained in 175 mm funnel. Return first 50 ml of filtrate to the filter. Collect filtrate until 3 hours shall have elapsed from time the  $\text{H}_2\text{O}$  and ground malt were first mixed. Prevent evaporation during filtration period as far as possible by placing a watch-glass over funnel and some suitable cover around stem of funnel, resting on neck of receiver.

Immediately dilute 20 ml of above infusion to 100 ml at  $20^\circ$ , transfer 10 ml of this infusion to 200 ml volumetric flask, and bring to  $20^\circ$ . If diastatic power of malt being examined is  $135^\circ$  Lintner or above, make (or repeat) determination, using 250 ml volumetric flask at this point and 200 ml of the buffered starch soln. Multiply diastatic power as computed under 49 by 1.25. Add 100 ml of buffered starch soln from a fast flowing pipet at  $20^\circ$ . Mix solns by rotating flask during the addition.

Maintain the "starch-infusion" mixture at  $20^{\circ} (\pm 0.2^{\circ})$  exactly 30 min. after addition of starch soln was begun. Add 10 ml of 0.5 *N* NaOH rapidly for each 100 ml of starch soln and mix thoroly by whirling flask. Make to mark at  $20^{\circ}$  and mix well.

Boil 10 ml of the Fehling soln and 10 ml of  $H_2O$  in small flask with narrow neck (200 ml Erlenmeyer). Add from buret ca  $\frac{2}{3}$  of amount of above digested starch soln probably required and boil 15–20 seconds, rotating constantly. Remove from flame. If still decidedly blue, add more soln, boil ca 10 seconds, and again observe color. When blue color has been almost discharged, and after boiling gently ca 2 min., add 3 drops of a 1% aqueous methylene blue soln. Continue boiling and add more soln until 0.1 ml, or even 1 drop, upon boiling, discharges the blue color. (Color becomes violet-lavender as end point nears.)

Repeat titration, adding at once almost whole amount of digested starch required and proceed to end point as directed. Let amount of digested starch soln required to reach end point in this second titration be called *A*. Interrupt boiling as little as possible after indicator has been added, so that flask remains filled with steam, preventing much access of air. Upon cooling the blue color usually returns.

49

## BLANK CORRECTION

Prepare blank by proceeding exactly as described under 48, except to add the NaOH to malt infusion before adding the starch soln. Add to 10 ml of the Fehling soln and 10 ml of  $H_2O$  a volume of this blank equal to final volume of digested starch soln required in above determination. Boil and again determine end point, using the *digested* starch soln, as directed under 48. Let amount of digested starch soln used here be called *B*.

*Corrected* diastatic power =  $\frac{4000}{A} \times \frac{B}{A}$ , in which  $\frac{4000}{A}$ , apparent diastatic power, is

modified by the fraction representing ratio of the blank titration to the original titration, which measures influence of the starch in the determination. To convert this to "dry basis," divide figure so found by (100 minus per cent moisture). Report as degrees Lintner (dry basis).

### PREPARED CORN OR RICE PRODUCTS (FLAKED CORN OR FLAKED RICE)—TENTATIVE

50

## MOISTURE

Weigh ca 5 g in glass weighing bottle or Al dish with tightly fitting cover, 40 mm in diameter, and dry at  $103\text{--}104^{\circ}$  4 hours. Record results to nearest first decimal

51

## FAT

Extract sample from moisture determination with anhydrous ether 5–6 hours, distil off ether, and dry in oven 1 hour. Cool, and weigh.

52

## EXTRACT

To 30 g of finely ground barley-malt contained in weighed mash beaker, add 200 ml of  $H_2O$  at  $46^{\circ}$ . Mix malt and  $H_2O$  with glass rod, washing off rod and sides of beaker with a little  $H_2O$ . Maintain temp. of  $45^{\circ}$  with constant stirring, preferably in mashing machine, 30 min. If no mashing apparatus is available, place beaker on wire screen contained in water bath. Raise temp.  $1^{\circ}$  per min. until  $67^{\circ}$  is reached. Add 20 g of the finely ground flaked corn or rice product, mix thoroly, and hold 30 min. at  $67^{\circ}$ . Warm up to  $70^{\circ}$  in 6 min. Hold at this temp. until saccharified,

testing every 3 min. by taking small portion of mash with thin glass rod and placing this in the cavities of a porcelain plate together with a drop of 0.01 *N* I soln, 42. A yellow coloration indicates complete conversion. Note time after reaching 70° until mash is completely inverted. Hold at 70° for a total of 60 min. Cool to room temp., remove beaker from bath, wash off thermometer or stirrer with a little H<sub>2</sub>O, dry beaker, and adjust contents to 450 g. Stir contents of beaker thoroly and filter thru fluted filter. Pour first 100 ml of filtrate back on filter, collecting entire filtrate in 500 ml Erlenmeyer flask. Determine sp. gr. with Reischauer or other pycnometer at 20° and find corresponding extract in the Plato Table, XLIII, 3.

Calculate extract as follows:

$$\text{Total extract} = \frac{\text{Extract} \times (800 + W \text{ in } 60 \text{ g of malt} + W \text{ in } 40 \text{ g of flakes})}{100 - \text{Extract}}, \text{ in}$$

which

Extract = extract from Plato's table;

*W* = moisture;

$$\text{Extract in flakes} = \frac{\text{Total extract} - \text{extract in } 60 \text{ g of malt}}{40} \times 100.$$

### CORN GRITS, CORN MEAL, BREWERS' RICE<sup>1</sup>—TENTATIVE

53

#### PREPARATION OF SAMPLE

If necessary, grind to fairly fine consistency.

54

MOISTURE.—See 50.

55

FAT.—See 51.

56

#### EXTRACT

The extract and moisture content of malt used must be known.

Boil 30 min. 20 g of finely ground sample in mash beaker with 200 ml of H<sub>2</sub>O, stirring with glass rod and replacing the evaporated H<sub>2</sub>O. Cool to 46°, and add 30 g of crushed malt. Mix, and wash off glass rod and sides of beaker with a little H<sub>2</sub>O. Maintain temp. of 45° 30 min. with constant stirring, preferably in mashing apparatus. If no mashing apparatus is available, place beaker on wire screen contained in water bath. Raise temp. 1° every minute until 70° is reached, and hold at this temp. until saccharified, testing every 3 min. by taking a small portion of the mash with thin glass rod and placing this in the cavities of a porcelain plate together with a drop of 0.01 *N* I soln, 42. A yellow coloration indicates complete inversion. Note time after reaching 70° until mash is converted. Hold at 70° for a total of 30 min. Cool to room temp., remove beaker from bath, wash off stirrer and thermometer with a little H<sub>2</sub>O, dry beaker, and adjust contents of beaker to 450 g. Stir with glass rod and filter thru fluted filter paper. Pour first 100 ml of filtrate back on filter, collecting entire filtrate in 500 ml Erlenmeyer flask. Determine sp. gr. with Reischauer or other pycnometer at 20° and find corresponding extract in the Plato Table, XLIII, 3. Calculate extract as directed under 52.

57

#### REFINED GRITS AND REFINED FLAKES

Proceed as directed for examination of Corn Grits except to boil 5 min. only in determining extract. See 53–56.

#### SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., 19, 76 (1936).

<sup>2</sup> Ibid., 22, 65, 73, 207 (1939).

## XV. WINES

1

### PHYSICAL EXAMINATION—TENTATIVE

Note and record following: (1) Whether container is "bottle full"; (2) appearance of wine, whether bright or turbid and whether there is any sediment; (3) condition when opened, whether still, gaseous, or carbonated; (4) color and depth of color; (5) odor, whether vinous, foreign or acetous, and (6) taste, whether dry, sweet, vinous, foreign, or acetous.

2

### PREPARATION OF SAMPLE—OFFICIAL

Remove any gas in wine by pouring sample back and forth in beakers. Filter wine, regardless of appearance. Determine immediately sp. gr. and those ingredients that are subject to change, such as alcohol, sugars, acids.

3

### SPECIFIC GRAVITY

Determine sp. gr. at 20/20° by means of a pycnometer as directed under XIV, 3, or by means of small accurately graduated hydrometer.

4

### ALCOHOL—OFFICIAL

(a) *By volume*.—Measure 100 ml of the liquid into 300–500 ml distillation flask, noting temp., and add 50 ml of H<sub>2</sub>O. Attach flask to vertical condenser by means of bent tube, distil almost 100 ml, and make to volume of 100 ml at same temp. (Foaming which sometimes occurs, especially with young wines, may be prevented by addition of small quantity of tannin.) To determine alcohol in wines that contain an abnormal quantity of acetic acid, add amount of normal NaOH soln necessary to exactly neutralize portion taken (calculated from acidity determination, 22) before proceeding with distillation (unnecessary for wines of normal taste and odor). Determine sp. gr. of distillate as directed under XIV, 3, at room temp. if desired, and obtain corresponding percentage of alcohol by volume from XLIII, Table 19.

(b) *By weight*.—From Table 21 obtain % alcohol by weight in distillate corresponding to % alcohol by volume, multiply by sp. gr. of distillate, and divide by sp. gr. of sample.

(c) *By immersion refractometer*.—Verify percentages of alcohol (a) and (b) by ascertaining immersion refractometer reading of distillate and obtaining corresponding percentages of alcohol from XLIII, Table 20.

### GLYCEROL IN DRY WINES

At no time during any of the evaporations should area of dish exposed to bath be greater in circumference than that covered by liquid in dish (easily done by allowing dish to float in bath).

5

### *Method I. By Direct Weighing—Official*

Evaporate 100 ml of the wine in porcelain dish on water bath maintained at 85–90° to ca 10 ml. Treat residue with ca 5 g of fine sand and 4–5 ml of milk of lime (containing 15 g of CaO per 100 ml) for each g of extract present and evaporate almost to dryness. Treat moist residue with 50 ml of alcohol, 90% by volume; remove substance adhering to sides of dish with spatula, and rub whole mass to a paste. Heat mixture on water bath, with constant stirring, to incipient boiling and decant liquid thru filter into small flask. Wash residue repeatedly by decantation

with 10 ml portions of hot 90% alcohol until filtrate amounts to ca 150 ml. Evaporate filtrate to a sirupy consistency in porcelain dish, transfer residue to small, glass-stoppered, graduated cylinder with 20 ml of absolute alcohol, and add 3 portions of 10 ml each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru filter, and wash cylinder and filter with mixture of 2 parts of absolute alcohol to 3 parts of anhydrous ether, also pouring wash liquor thru filter. Evaporate filtrate to a sirupy consistency, dry 1 hour at 98–100°, weigh, ignite, and weigh again. Loss on ignition = weight of glycerol.

6 *Method II. By Oxidation with Dichromate—Official*

Evaporate 100 ml of wine in porcelain dish on water bath maintained at 85–90° to ca 10 ml. Treat residue with ca 5 g of fine sand and 4–5 ml of milk of lime (containing 15 g of CaO per 100 ml). Proceed as directed under XXXIII, 74, beginning, "evaporate almost to dryness, with frequent stirring," except to dilute the soln of glycerol after treatment with  $\text{Ag}_2\text{CO}_3$  and Pb acetate to volume of 100 ml instead of 50 ml. Observe precautions given concerning temp. at which all evaporations are to be made.

7 GLYCEROL IN SWEET WINES—OFFICIAL

With wines in which the extract exceeds 5 g per 100 ml, heat 100 ml to boiling in flask and treat with successive small portions of milk of lime until wine becomes first darker and then lighter in color. Cool, add 200 ml of alcohol, allow the precipitate to subside, filter, and wash with alcohol. Treat the combined filtrate and washings as directed under 5 or 6.

8 GLYCEROL-ALCOHOL RATIO—OFFICIAL

Express this ratio as  $X:100$ , in which  $X$  is obtained by multiplying percentage weight of glycerol by 100 and dividing result by percentage of alcohol by weight.

EXTRACT

9 *Method I. From Specific Gravity of Dealcoholized Wine—Official*

Calculate sp. gr. of the dealcoholized wine,  $D$ , by following formula:

$$D = S + 1 - A.$$

$$S = \text{sp. gr. of sample, } 3;$$

$$A = \text{sp. gr. of alcoholic distillate, } 4(a).$$

From Table 3, under XLIII, ascertain percentage by weight of extract in the dealcoholized wine corresponding to value of  $D$ . This figure  $\times$  the value of  $S = \text{g of extract per 100 ml of wine.}$

10 *Method II. By Evaporation—Official*

(a) *In dry wines, extract content of less than 3 g per 100 ml.*—In 75 ml flat-bottomed Pt dish, ca 85 mm in diameter, evaporate 50 ml of sample on water bath to sirupy consistency. Heat residue 2–5 hours in drying oven at temp. of boiling  $\text{H}_2\text{O}$ , cool in desiccator, and weigh as soon as dish and contents reach room temp.

(b) *In sweet wines.*—If extract content is 3–6 g per 100 ml, treat 25 ml of sample as directed under (a). If extract exceeds 6 g per 100 ml, accept result obtained as directed under 9, and attempt no gravimetric determination because of inaccurate results obtained by drying levulose at a high temp.



**11 NON-SUGAR SOLIDS (SUGAR-FREE EXTRACT)—OFFICIAL**

Subtract quantity of reducing sugars before inversion, **12**, from extract, **9** or **10**. If sucrose is present, determine non-sugar solids by subtracting from the extract the sum of the reducing sugars before inversion and the sucrose.

**12 REDUCING SUGARS—OFFICIAL**

(a) *Dry wines*.—Place 200 ml of sample in porcelain dish, exactly neutralize with normal NaOH, calculating quantity required from determination of acidity, and evaporate to ca 50 ml. Transfer to 200 ml flask, add sufficient neutral Pb acetate soln to clarify, dilute to mark with H<sub>2</sub>O, shake, and pass thru folded filter. Remove the Pb with dry K oxalate and determine reducing sugars as directed under **XXXIV**, **38**.

(b) *Sweet wines*.—Approximate sugar content by subtracting **2** from the extract and use such a quantity of the sample that the aliquot taken for the Cu reduction contains not over 240 mg of invert sugar. Proceed as directed under (a).

**SUCROSE****13 I. By Reducing Sugars Before and After Inversion—Official**

Proceed as directed under **XXXIV**, **29**, using method given under **XXXIV**, **38**, for the determination of reducing sugars.

**14 II. By Polarization—Official**

Polarize before and after inversion in 200 mm tube, as directed under **XXXIV**, **23** or **24**, a portion of filtrate obtained under **12**. In calculating percentage of sucrose do not fail to take into consideration relation of weight of sample contained in 100 ml to the normal weight for the instrument.

**15 COMMERCIAL GLUCOSE—OFFICIAL**

Polarize a portion of filtrate obtained under **12**, after inversion in 200 mm jacketed tube at 87°, as directed under **XXXIV**, **30**. In calculating percentage of glucose do not fail to take into consideration relation of weight of sample contained in 100 ml to the normal weight for the instrument.

**16 ASH—OFFICIAL**

Proceed as directed under **XXXIV**, **9** or **10**, using residue from 50 ml of the wine. Char carefully (decrepitation), and do not exceed 550° during ashing.

**17 ALKALINITY OF WATER-SOLUBLE ASH—OFFICIAL**

Extract ash obtained as directed under **16** with successive small portions of hot H<sub>2</sub>O until filtrate amounts to ca 60 ml and proceed as directed under **XXXIV**, **14**. Express alkalinity in terms of ml of 0.1 *N* acid required to neutralize the water-soluble ash from 100 ml of the wine.

**18 ALKALINITY OF WATER-INSOLUBLE ASH—OFFICIAL**

Ignite filter and residue from **17** in the Pt dish in which the wine was ashed and proceed as directed under **XXXIV**, **15**. Express alkalinity in terms of ml of 0.1 *N* acid required to neutralize the water-insoluble ash from 100 ml of the wine.

**19 PHOSPHORIC ACID—OFFICIAL**

Dissolve the ash, **16**, in 50 ml of boiling HNO<sub>3</sub> (1+9), filter, wash paper, and determine P<sub>2</sub>O<sub>5</sub> in combined filtrate and washings as directed under **II**, **9** or **12**. If

ash ignites without difficulty, no free phosphoric acid need be suspected. If any free acid is present, ash remains black even after repeated leaching. In latter case, add Ca acetate or mixture containing 3 parts of  $\text{Na}_2\text{CO}_3$  and 1 part of  $\text{NaNO}_3$  to avoid loss of  $\text{P}_2\text{O}_5$  before attempting to ash.

20

## SULFURIC ACID—OFFICIAL

Precipitate directly  $\text{H}_2\text{SO}_4$  in 50 ml of sample by means of 10%  $\text{BaCl}_2$  soln after acidifying with small excess of  $\text{HCl}$ , and determine resulting  $\text{BaSO}_4$  as directed under **XII, 27**. Allow precipitate to stand for at least 6 hours before filtering. Report as  $\text{SO}_3$ , using factor 0.3430.

21

## CHLORIDES—OFFICIAL

To 100 ml of dry wine or 50 ml of sweet wine, add sufficient  $\text{Na}_2\text{CO}_3$  to make distinctly alkaline. Evaporate to dryness, ignite at heat not above  $550^\circ$ , cool, extract residue with hot  $\text{H}_2\text{O}$ , acidify the  $\text{H}_2\text{O}$  extract with  $\text{HNO}_3$  (1+4), and determine chlorides as directed under **XII, 35** or **37**.

22

## ACIDITY—OFFICIAL

(a) *Phenolphthalein*.—In large porcelain dish neutralize ca 250 ml of recently boiled  $\text{H}_2\text{O}$  with 0.1 *N* alkali, using ca 2 ml of phenolphthalein indicator soln. Quantity of sample to be used depends on depth of color of wine; it is generally 5 ml for deeply colored red wine and 20 ml for white wine. Titrate rapidly to distinct pink. Heat the portion of wine to be titrated to incipient boiling to remove  $\text{CO}_2$  (all wines, still or gaseous) and transfer it to dish with portion of the neutralized  $\text{H}_2\text{O}$ .

(b) *Azolitmin*.—Measure 20 ml of the wine into 250 ml beaker, heat rapidly to incipient boiling, and immediately titrate with 0.1 *N*  $\text{NaOH}$  soln. Determine end point with neutral 0.05% azolitmin soln as outside indicator. Place indicator in cavities of spot plate and spot the wine into the azolitmin soln. End point is reached when color of indicator remains unchanged by addition to wine of a few drops of 0.1 *N* alkali.

(c) *Phenolphthalein powder* (artificially colored wines).—Into cavities of a spot plate place mixture of one part of phenolphthalein and 100 parts of dry powdered  $\text{K}_2\text{SO}_4$  and spot the wine into the powder. (End of titration is indicated when powder acquires pink tint. Powder should not be too fine. Addition of neutral alcohol to the wine will facilitate flow of the wine into the powder.) Tilt spot plate and allow wine to flow into powder from tip of a heavy stirring rod.

Express results in terms of tartaric acid. 1 ml of 0.1 *N*  $\text{NaOH}$  soln = 0.0075 g of tartaric acid.

## TOTAL VOLATILE ACIDITY

23

*Method I—Official*

Heat rapidly to incipient boiling 50 ml of the wine in 500 ml distillation flask and pass steam thru until 15 ml of distillate requires only 2 drops of 0.1 *N*  $\text{NaOH}$  soln for neutralization. Boil the  $\text{H}_2\text{O}$  used to generate the steam several minutes before connecting steam generator with distillation flask in order to expel  $\text{CO}_2$ . Titrate rapidly with 0.1 *N*  $\text{NaOH}$  soln, using phenolphthalein indicator. (Color should remain ca 10 seconds.) Express results as acetic acid. 1 ml of 0.1 *N*  $\text{NaOH}$  soln = 0.0060 g of acetic acid.

24

*Method II—Official*

Introduce 10 ml of the wine, freed from excess  $\text{CO}_2$  by pouring back and forth

between large beakers, into inner tube of a modified Hortvet type of distillation apparatus (e.g., Fig. 20). (Preferably use a sufficiently large inner Sellier tube (ca  $1\frac{1}{2} \times 8$ ") and a large distillation trap.) Connect with a slanting or vertical straight tube condenser and distil, by heating the outer flask, into a 300 ml Erlenmeyer flask (marked at ca 80 ml capacity), until 80 ml of distillate has been obtained. If the wine is new or is charged with  $\text{CO}_2$ , bring distillate to boiling, boil 30 seconds, and titrate hot with 0.1 *N* NaOH soln, using phenolphthalein indicator. As an alternative, adjust  $\text{H}_2\text{O}$  flow thru condenser so that condensate is received hot. Distil at such a rate as to obtain the 80 ml in ca 10 min. For wines with abnormally high acetic acid content, continue distillation and titrate each succeeding 10 ml of distillate until not more than 1 drop of standard alkali is required to reach neutral point.

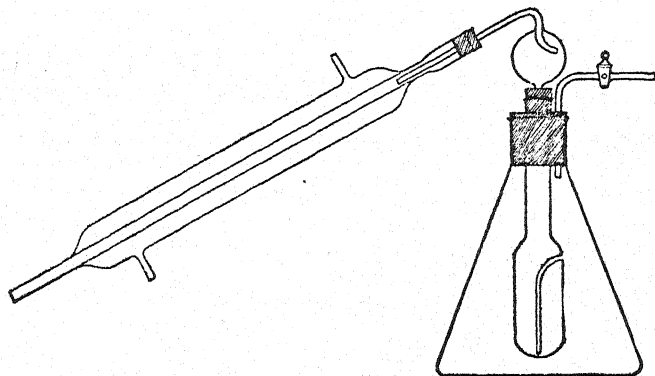


FIG. 20.—APPARATUS FOR DETERMINATION OF VOLATILE ACIDITY

If the wine is free of  $\text{CO}_2$ , or has been previously freed from  $\text{CO}_2$  by heating to incipient boiling and cooling or by shaking thoroly in vacuo in a flask connected to water aspirator, the distillate may be titrated cold. Use a 10 ml buret graduated in 1/20 or 1/50 ml.

#### VOLATILE ACIDITY—EXCLUSIVE OF $\text{SO}_2$

25

##### *Method I—Tentative*

Immediately after neutralization, if necessary, cool distillate **23** or **24**, to room temp. by plunging in an ice bath; add rapidly to 100 ml of distillate from a graduated cylinder 5 ml. of  $\text{H}_2\text{SO}_4$  (1+3) and several ml of starch indicator; and titrate with 0.1 *N* iodine, using 10 ml buret. Subtract ml of 0.1 *N* iodine used from the ml of 0.1 *N* NaOH used, **23** or **24**, and express results as g of acetic acid per 100 ml of wine.

26

##### *Method II—Tentative*

Pipet 50 ml of wine into 100 ml volumetric flask. If white, add 2–3 drops of phenolphthalein indicator and neutralize to decided pink by a clear saturated soln of  $\text{Ba}(\text{OH})_2$ ; if red, add sufficient  $\text{Ba}(\text{OH})_2$  to bring mixture to ca pH 8, using phenolphthalein as external indicator. Allow mixture to stand 30 min. and maintain at the phenolphthalein end point by the addition of more  $\text{Ba}(\text{OH})_2$  if necessary. Make up to 100 ml, mix, and filter rapidly thru fluted, rapidly filtering paper (such as Whatman's No. 2). Pipet 20 ml of filtrate into inner Sellier tube of a Hortvet type apparatus, using larger type tube; add 1 ml of  $\text{H}_2\text{SO}_4$  (1+3), and distil over 100 ml. Titrate with 0.1 *N* NaOH soln, using phenolphthalein indicator.

27

## FIXED ACIDITY—OFFICIAL

Calculate fixed acidity as tartaric by multiplying total volatile acidity by 1.25 and subtracting this product from total acidity.

28

TOTAL TARTARIC ACID<sup>2</sup>—OFFICIAL

Neutralize 100 ml of sample with *N* NaOH soln, calculating from acidity, 22, number of ml of *N* alkali necessary. If more than 10 ml of alkali is added, evaporate to ca 100 ml. Add to neutralized soln 0.075 g of tartaric acid for each ml of *N* alkali added. It is essential that the tartaric acid be pure; if necessary recrystallize. After the tartaric acid has dissolved add 2 ml of glacial acetic acid and 15 g of KCl. After the KCl has dissolved, add 15 ml of alcohol, stir vigorously until the K bitartrate begins to precipitate, and let stand in icebox at 15–18° at least 15 hours. Decant the liquid on Gooch crucible prepared with very thin film of asbestos, or on filter paper in Büchner funnel. Wash precipitate from beaker with filtrate (keep cold) and finally rinse beaker and filter 3 times with a few ml of a mixture of 15 g of KCl, 20 ml of alcohol, and 100 ml of H<sub>2</sub>O, using not more than 20 ml of the wash soln in all. Transfer the asbestos or paper and precipitate to beaker in which precipitation was made; wash Gooch crucible or Büchner funnel with hot H<sub>2</sub>O, using ca 50 ml in all; heat to boiling and titrate the hot soln with 0.1 *N* NaOH soln, using phenolphthalein indicator. Increase number of ml of 0.1 *N* alkali required by 1.5 ml to allow for solubility of precipitate. Under these conditions 1 ml of 0.1 *N* alkali = 0.015 g of tartaric acid. To obtain grams of total tartaric acid per 100 ml of the wine, subtract the quantity of tartaric acid added from this result.

29

FREE TARTARIC ACID AND CREAM OF TARTAR<sup>2</sup>—OFFICIAL

Calculate in following manner:

*A* = total tartaric acid, 28, divided by 0.015;

*B* = total alkalinity of ash (*C* + *D*);

*C* = alkalinity of water-soluble ash, 17; and

*D* = alkalinity of water-insoluble ash, 18.

Then

(1) If *A* is greater than *B*,

Cream of tartar =  $0.0188 \times C$ , and

Free tartaric acid =  $0.015 \times (A - B)$ ;

(2) If *A* equals *B* or is smaller than *B* but greater than *C*,

Cream of tartar =  $0.0188 \times C$ , and

Free tartaric acid = 0; and

(3) If *A* is smaller than *C*,

Cream of tartar =  $0.0188 \times A$ , and

Free tartaric acid = 0.

30

## CITRIC AND MALIC ACIDS—TENTATIVE

For citric and malic acids occurring in normal wines in small quantities only, use 100 ml of sample and evaporate to 45 ml. After saponification proceed as directed under XXVI, 31 or 34.

## TANNIN AND COLORING MATTER—OFFICIAL

31

## REAGENTS

(a) *Oxalic acid soln.*—0.1 *N*. 1 ml = 0.00416 g of tannin.

(b) *Standard potassium permanganate soln.*—Dissolve 1.333 g of KMnO<sub>4</sub> in 1 liter of H<sub>2</sub>O and standardize soln against (a).

(c) *Indigo soln.*—Dissolve 6 g of Na indigotin disulfonate in 500 ml of H<sub>2</sub>O by heating; cool, add 50 ml of H<sub>2</sub>SO<sub>4</sub>, make up to 1 liter, and filter.

(d) *Purified boneblack.*—Boil 100 g of finely powdered boneblack with successive portions of HCl (1+3), filter, and wash with boiling H<sub>2</sub>O until free from chlorides. Keep covered with H<sub>2</sub>O.

32

DETERMINATION<sup>4</sup>

Dealcoholize 100 ml of the wine by evaporation and dilute with H<sub>2</sub>O to original volume. Transfer 10 ml to a 2 liter porcelain dish and add ca 1 liter of H<sub>2</sub>O and exactly 20 ml of the indigo soln. Add the standard KMnO<sub>4</sub> soln, 1 ml at a time, until the blue color changes to green; then add a few drops at a time until the color becomes golden yellow. Designate number of ml of KMnO<sub>4</sub> soln as "a."

Treat 10 ml of the prepared dealcoholized wine 15 min. with boneblack, filter, and wash thoroly with H<sub>2</sub>O. Add 1 liter of H<sub>2</sub>O and 20 ml of the indigo soln and titrate with KMnO<sub>4</sub>, as directed above. Designate number of ml of KMnO<sub>4</sub> used as "b."

Then  $a - b = c$ , number of ml of the KMnO<sub>4</sub> soln required for oxidation of the tannin and coloring matter in 10 ml of the wine.

33

## CRUDE PROTEIN—OFFICIAL

Determine N in 50 ml of the wine as directed under II, 21, 22, or 23, and multiply result by 6.25.

34

## PENTOSANS—OFFICIAL

(Applicable to dry wines only.)

Proceed as directed under XXVII, 36, except to use 100 ml of the wine and 43 ml of HCl in beginning distillation. (Owing to interference of sugars this determination can be made in dry wines only.)

35

## GUM AND DEXTRIN—TENTATIVE

Evaporate 100 ml of the wine to ca 10 ml and add 10 ml of alcohol. If gum or dextrin is present (indicated by formation of voluminous precipitate), continue addition of alcohol, slowly and with stirring, until 100 ml has been added. Let stand overnight, filter, and wash with alcohol, 80% by volume. Dissolve precipitate on the paper with hot H<sub>2</sub>O, hydrolyze filtrate and washings with HCl, and proceed as directed under XXVII, 30.

36

## NITRATES—TENTATIVE

(a) *White wine.*—Treat a few drops of the wine in porcelain dish with 2-3 ml of H<sub>2</sub>SO<sub>4</sub> that contains ca 0.1 g of diphenylamine<sup>5</sup> per 100 ml. The deep blue color formed in presence of nitrates appears so quickly that it is not obscured, even in sweet wine, by blackening produced by action of H<sub>2</sub>SO<sub>4</sub> on the sugar.

(b) *Red wine.*—Clarify with basic Pb acetate, filter, remove Pb from filtrate with Na<sub>2</sub>SO<sub>4</sub>, filter again, and treat a few drops of this filtrate as directed under (a).

37

## COLORING MATTERS—TENTATIVE.—See XXI.

38

## SULFUROUS ACID—TENTATIVE

Proceed as directed under XXXII, 32, except to dilute 100 ml of the wine with 200 ml of S-free H<sub>2</sub>O and add mixture to flask thru dropping funnel, followed by 20 ml of conc. HCl. Allow mixture to stand a few minutes until fumes have settled.

Adjust burner so that vapors rise no higher than one-tenth the length of water jacket of condenser and boil sample 90 min. Adjust flow of  $\text{CO}_2$  so that a slow, but steady stream passes thru receiver during distillation and complete analysis as directed in XXXII, 32. Report results as mg of  $\text{SO}_2$  per liter.

39

## PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII. The determination of  $\text{SO}_2$  should be quantitative. Occurrence of small quantities of salicylic acid in the grape has been reported in the literature and for that reason not more than 50 ml of sample should be used in testing for that preservative. Concord grapes contain 3.2 mg of salicylic acid per 100 ml of juice.<sup>6</sup>

## SELECTED REFERENCES

<sup>1</sup> J. Ind. Eng. Chem., 1, 31 (1909); J. Assoc. Official Agr. Chem., 22, 210 (1939).

<sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull. 162, p. 72.

<sup>3</sup> Ibid., p. 75.

<sup>4</sup> Ann. Oenologie, 2, 1 (1871-72).

<sup>5</sup> Arch. Hyg., 2, 273 (1884).

<sup>6</sup> J. Am. Chem. Soc., 25, 242 (1903).

## XVI. DISTILLED LIQUORS

### SPIRITS

1

#### PHYSICAL EXAMINATION—TENTATIVE

Note and record following: (1) color and depth of color; (2) odor—whisky, brandy, rum, etc.; or foreign; (3) taste—whiskey, brandy, rum, etc., or foreign.

2

#### SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° by means of pycnometer as directed under XIV, 3, or by means of small, accurately graduated hydrometer.

3

#### ALCOHOL BY WEIGHT—OFFICIAL

Weigh 20–25 g of sample into distillation flask, dilute with 100 ml of H<sub>2</sub>O, and distil nearly 100 ml. Weigh distillate or make to volume at room temp., noting temp. In either case determine sp. gr. as directed under XIV, 3, at room temp. if desired. Obtain corresponding percentage of alcohol by weight from Tables 19 and 21, XLIII, multiply this figure by weight of distillate, and divide by weight of sample taken.

The alcohol content of distillate may be checked by determining immersion refractometer reading and obtaining percentage of alcohol from Table 20, under XLIII.

#### ALCOHOL BY VOLUME

4

##### *Method I—Official*

From sp. gr. of distillate obtained under 3 ascertain corresponding percentage of alcohol by volume from XLIII, Table 19. Multiply this figure by volume of distillate and divide by volume of sample (calculated from sp. gr.) to obtain percentage of alcohol by volume in original sample.

5

##### *Method II—Official*

Measure 25 ml of sample into distillation flask, noting temp., dilute with 100 ml of H<sub>2</sub>O, distil nearly 100 ml, make to volume at same temp., and determine sp. gr. as directed under XIV, 3. Obtain, from Table 19, XLIII, the corresponding percentage of alcohol by volume in distillate and multiply by 4 to obtain percentage of alcohol by volume in original substance.

The alcohol content of distillate may be checked by determining immersion refractometer reading and obtaining percentage of alcohol from Table 20, XLIII.

6

#### EXTRACT—OFFICIAL

Weigh, or measure at 20°, 25–100 ml of sample, evaporate to dryness on steam bath, transfer to water oven, and dry at temp. of boiling H<sub>2</sub>O 1 hour.

7

#### ASH—OFFICIAL

Proceed as directed under XXXIV, 9 or 10, using residue from 6.

8

#### TOTAL ACIDS—OFFICIAL, FIRST ACTION

Neutralize ca 250 ml of boiled H<sub>2</sub>O in a porcelain evaporating dish (7½" dish is convenient). Add 25 ml of sample and titrate with 0.1 N NaOH soln, using ca 2 ml of phenolphthalein indicator soln.

9

## FIXED ACIDS—OFFICIAL, FIRST ACTION

Evaporate 25–50 ml of sample to dryness in a Pt dish on steam bath and dry 1 hour in oven at 100°. Dissolve, and transfer residue with several portions of neutral alcohol of approximately the same proof as sample, using 25–50 ml in all, to a porcelain dish containing ca 250 ml of neutralized boiled H<sub>2</sub>O. Titrate with 0.1 N NaOH, using a 10 ml buret graduated in 0.05 ml and same amount of indicator as under 8.

10

## VOLATILE ACIDS—OFFICIAL, FIRST ACTION

Volatile acids = total acids – fixed acids.

11

## ESTERS—OFFICIAL

Measure 100–200 ml of sample into distillation flask, add 12.5–25 ml of H<sub>2</sub>O, and distil slowly 100–200 ml, depending upon amount of sample taken, using mercury valve to prevent loss of alcohol. Exactly neutralize free acid in 50 ml of distillate with 0.1 N alkali and add measured excess of 25–50 ml of 0.1 N alkali. Then either boil an hour under reflux condenser, cool, and titrate with 0.1 N acid, or allow soln to stand overnight in stoppered flask with the excess of alkali, heat with tube condenser 30 min. at temp. below the b.p., cool, and titrate. Calculate ml of 0.1 N alkali used in saponification of the esters as ethyl acetate. 1 ml of 0.1 N alkali = 0.0088 g of ethyl acetate. Run blank, using H<sub>2</sub>O in place of distillate, and make any necessary correction.

## ALDEHYDES

*Colorimetric Method—Official*

12

## REAGENTS

(a) *Aldehyde-free alcohol*.—Redistil alcohol over NaOH or KOH, add 2–3 g per liter of metaphenylenediamine hydrochloride, digest at ordinary temp. several days (or under reflux condenser on steam bath several hours), and distil slowly, rejecting first 100 ml and last 200 ml of distillate.

(b) *Sulfite-fuchsin soln*.—Dissolve 0.50 g of pure fuchsin in 500 ml of H<sub>2</sub>O, add 5 g of SO<sub>2</sub> dissolved in H<sub>2</sub>O, make up to 1 liter, and allow to stand until colorless. As this soln decomposes rapidly, prepare it in small quantities and keep at low temp.

(c) *Standard acetaldehyde soln*.—Prepare according to directions of Vasey,<sup>1</sup> as follows: Grind aldehyde ammonia in mortar with anhydrous ether and decant the ether. Repeat this operation several times and dry purified salt in current of air and then in vacuo over H<sub>2</sub>SO<sub>4</sub>. Dissolve 1.386 g of this purified aldehyde ammonia in 50 ml of alcohol, add 22.7 ml of N alcoholic H<sub>2</sub>SO<sub>4</sub>, make up to 100 ml, and add 0.8 ml of alcohol for the volume of the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitate. Allow mixture to stand overnight, and filter. This soln contains 1 g of acetaldehyde in 100 ml and will retain its strength.

The standard found most convenient for use is 2 ml of this strong aldehyde soln diluted to 100 ml with alcohol, 50% by volume. 1 ml of this soln = 0.0002 g of acetaldehyde. Make up soln every day or so, as it loses strength.

13

## DETERMINATION

Determine aldehyde in prepared distillate, 11. Dilute 5–10 ml of distillate to 50 ml with aldehyde-free alcohol, 50% by volume; add 25 ml of the sulfite-fuchsin soln, and allow to stand 15 min. at 15°. Solns and reagents should be at 15° when they are mixed. Prepare standards of known strength and blanks in same way.



Comparison standards found most convenient for use contain 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg of acetaldehyde.

14

*Volumetric Method<sup>2</sup>—Official, First Action*

## REAGENTS

(a) *0.05 N sodium thiosulfate soln.*—Standardize against 0.05 N  $K_2Cr_2O_7$  soln as follows: Place 20 ml of 0.05 N  $K_2Cr_2O_7$  soln in glass-stoppered flask and add 5 ml 15% KI soln. Add 2.5 ml of HCl and dilute with 100 ml of  $CO_2$ -free  $H_2O$ , then immediately titrate the liberated I with the thiosulfate soln until yellow color has almost disappeared; add 1–2 ml of starch indicator, and continue, with constant shaking, addition of thiosulfate soln until blue color just disappears.

(b) *Iodine soln.*—0.05 N. Standardize this soln against the thiosulfate soln.

(c) *Sodium bisulfite soln.*—Prepare ca 0.05 N soln. (This soln will not deteriorate so fast if it contains 5–10% of alcohol; it should not be used after 2–3 days.)

15

## DETERMINATION

Pipet 50 ml of sample into an Erlenmeyer flask and add 10 ml of  $H_2O$  and a few small pieces of carborundum to insure even boiling. Distil 50 ml or slightly more into a glass-stoppered flask, using a delivery tube immersed in 100 ml of boiled  $H_2O$  ( $CO_2$ -free). Using a pipet, add 25 ml of the bisulfite soln and allow to stand ca 30 min., shaking occasionally. Add excess (ca 30 ml) of the I soln, titrate this excess with the thiosulfate soln, and calculate as acetaldehyde. 1 ml of 0.05 N soln = 0.0011 g of acetaldehyde. The difference between the two thiosulfate titrations (ml of 0.05 N bisulfite used)  $\times 0.0011$  = g of acetaldehyde.

NOTES: Do not add the starch indicator until the yellow color of the I soln has almost disappeared. As the end point is approached the soln will have a decided violet tint rather than a blue, as is customary with I and starch, if percentage of alcohol is high. If the end point is in doubt, add a little more of the starch indicator. The formation of a bluish-violet color indicates that the end point has not been reached. Always run a blank on the bisulfite soln with each series of aldehyde determinations, using same quantity of bisulfite and I soln as in determination of sample.

## FURFURAL—OFFICIAL

16

## REAGENT

*Standard furfural soln.*—Dissolve 1 g of redistilled furfural in 100 ml of 95% alcohol. Prepare standards by diluting 1 ml of this soln to 100 ml with alcohol, 50% by volume. 1 ml of this soln contains 0.1 mg of furfural. (The strong furfural soln will retain its strength, but the dilute soln will not.)

17

## DETERMINATION

Dilute 10–20 ml of the prepared distillate, 11, to 50 ml with furfural-free alcohol, 50% by volume. Add 2 ml of colorless aniline and 0.5 ml of HCl (sp. gr. 1.125) and keep 15 min. in water bath at ca 15°. Prepare standards of known strength and blanks in same way. The comparison standards found most convenient for use contain 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mg of furfural.

DETECTION OF ACETONE, KETONES, ISOPROPYL ALCOHOL,  
AND TERTIARY BUTYL ALCOHOL—TENTATIVE

18

## REAGENT

*Mercuric sulfate soln.*—Mix 5 g of yellow  $\text{HgO}$  with 40 ml of  $\text{H}_2\text{O}$ , add with stirring 20 ml of  $\text{H}_2\text{SO}_4$  and 40 ml of  $\text{H}_2\text{O}$ , and stir until completely dissolved.

19

## DETERMINATION

To 2 ml of distillate, 11, add 3 ml of  $\text{H}_2\text{O}$  and 10 ml of the mercuric sulfate soln. Heat on boiling water bath 3 min. A white or yellow precipitate forming within 3 min. indicates presence of any of above-mentioned compounds. Disregard any precipitate forming after 3 min. on boiling water bath.

## FUSEL OIL—OFFICIAL

20

## REAGENTS

(a) *Purified carbon tetrachloride.*—Mix in separatory funnel crude  $\text{CCl}_4$  with 1/10 its volume of  $\text{H}_2\text{SO}_4$ , shake thoroly at frequent intervals, and allow to stand overnight. Wash free of acid and impurities with tap  $\text{H}_2\text{O}$ , remove  $\text{H}_2\text{O}$ , add an excess of  $\text{NaOH}$  soln, and distil the  $\text{CCl}_4$ .

(The refuse  $\text{CCl}_4$  after titration is purified for further work by collecting in large bottle, adding  $\text{NaOH}$  soln (1+1), shaking, washing with tap  $\text{H}_2\text{O}$  until washings are neutral to phenolphthalein, and distilling.)

(b) *Oxidizing soln.*—Dissolve 100 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  in 900 ml of  $\text{H}_2\text{O}$  and add 100 ml of  $\text{H}_2\text{SO}_4$ .

21

## DETERMINATION

(1) To 50 ml of sample add 50 ml of  $\text{H}_2\text{O}$  then 20 ml of 0.5  $N$   $\text{NaOH}$ , and saponify mixture by boiling for an hour under reflux condenser; or, (2) mix 50 ml of the liquid and 50 ml of  $\text{H}_2\text{O}$  with 20 ml of 0.5  $N$   $\text{NaOH}$ , allow to stand overnight at room temp., and distil directly. Connect flask with distillation apparatus, distil 90 ml, add 25 ml of  $\text{H}_2\text{O}$ , and continue distillation until an additional 25 ml is collected.

Whenever aldehydes are present in excess of 15 parts per 100,000, add to distillate 0.5 g of metaphenylenediamine hydrochloride, boil under reflux condenser an hour, distil 100 ml, add 25 ml of  $\text{H}_2\text{O}$ , and continue distillation until an additional 25 ml is collected. Approximately saturate distillate with finely ground  $\text{NaCl}$  and add saturated  $\text{NaCl}$  soln until sp. gr. is 1.10. Extract this salt soln 4 times with the purified  $\text{CCl}_4$ , using 40, 30, 20, and 10 ml, respectively, and wash the  $\text{CCl}_4$  3 times with 50 ml portions of saturated  $\text{NaCl}$  soln, and twice with saturated  $\text{Na}_2\text{SO}_4$  soln. Transfer the  $\text{CCl}_4$  to flask containing 50 ml of the oxidizing soln and boil 8 hours under reflux condenser.

Add 100 ml of  $\text{H}_2\text{O}$  and distil until only ca 50 ml remains. Add 50 ml of  $\text{H}_2\text{O}$  and again distil until 35–50 ml is left. Use extreme care to prevent the oxidizing mixture from burning and baking on side of distilling flask. Distillate should be water white; if it is colored discard it and repeat determination. Titrate distillate with 0.1  $N$   $\text{NaOH}$ , using phenolphthalein indicator. 1 ml of 0.1  $N$   $\text{NaOH}$  = 0.0088 g of amyl alcohol. (It is preferable to use ground-glass connections thruout.)

Conduct blank determination upon 100 ml of  $\text{CCl}_4$ , beginning blank at that point of procedure immediately after extraction and just before washings with  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  solns.

## METHYL ALCOHOL

*Modified Denigès' Method<sup>3</sup>—Tentative*

## REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 3 g of  $\text{KMnO}_4$  and 15 ml of sirupy phosphoric acid (85%) in 100 ml of  $\text{H}_2\text{O}$ .

(b) *Oxalic-sulfuric acid soln.*—Dissolve 5 g of  $\text{H}_2\text{C}_2\text{O}_4$  in 100 ml of  $\text{H}_2\text{SO}_4$  (1+1).

(c) *Modified Schiff's reagent.*—Dissolve 0.2 g of Kahlbaum's rosaniline  $\text{HCl}$  in ca 120 ml of hot  $\text{H}_2\text{O}$ . Cool, and add 2 g of  $\text{Na}_2\text{SO}_3$  previously dissolved in 20 ml of  $\text{H}_2\text{O}$ . Add 2.0 ml of  $\text{HCl}$ , dilute soln to 200 ml, and place in the refrigerator at least 24 hours before using.

## PREPARATION OF SAMPLE

Make preliminary test as directed under 25, using an 0.5% standard to ascertain whether more than that quantity of methanol is present (necessary only on unknown samples suspected of being recovered alcohol denatured with methanol). If more than 0.5% methanol is present, dilute to that strength, or less, with ca 50% ethyl alcohol. Distil a 25 ml portion, using a fractionating column<sup>4</sup>, and collect ca 8.5 ml in ca 7 fractions, each consisting of ca 1.2 ml. Keep column under total reflux 30 min. before taking off first fraction, and reflux 15 min. between fractions. (This should produce a distillate containing ca 94% total alcohols.) Dilute distillate to 22% total alcohols and then make to any convenient volume with 22% ethyl alcohol.

## DETERMINATION

Transfer 0.25 ml of the diluted alcoholic soln to a 6" Nessler tube containing 4.75 ml of  $\text{H}_2\text{O}$ . Add 2 ml of the  $\text{KMnO}_4$  soln and allow to stand 10 min. with occasional shaking without inverting the tube, and then add 2 ml of the oxalic-sulfuric acid soln. Add 5 ml of the modified Schiff reagent, mix thoroly, by inverting tube 3 times, stopper, and allow to stand 1 hour. Compare depth of color with known standards analyzed at same time. Comparison standards found most convenient for use contain 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12% methyl alcohol by volume. Use 22% ethyl alcohol in comparison standards.

*Immersion Refractometer Method<sup>4</sup>—Official*

Determine Zeiss immersion refractometer reading at  $17.5^\circ$  of distillate obtained in determination of alcohol. If, on reference to the table, 27, the refractometer reading shows a sp. gr. agreeing with that obtained in alcohol determination, it may be assumed that no methyl alcohol is present. If, however, there is present an appreciable quantity of methyl alcohol, the low reading will at once indicate the fact. If the absence from the soln of refractive substances other than  $\text{H}_2\text{O}$  and the alcohols is assured, this difference in refraction is conclusive of presence of methyl alcohol.

The addition of methyl alcohol to ethyl alcohol decreases the refractive index in direct proportion to the quantity added; hence the quantitative calculation is made by interpolation in the table, 24, of the figures for pure ethyl and methyl alcohol of the same sp. gr. as the sample.

*Example.*—Distillate has sp. gr. at  $15.56^\circ$  of 0.9625 and refractometer reading at  $17.5^\circ$  of 43.1. By interpolation in the table, the readings for ethyl and methyl alcohol of this gravity are 65.2 and 31.7, respectively, and the difference is 33.5;  $65.2 - 43.1$

=22.1;  $(22.1 \div 33.5)100 = 66.0$ , showing that 66.0% of total alcohol present is methyl alcohol.

27 *Scale readings on Zeiss immersion refractometer at 17.5°, corresponding specific gravities of ethyl and methyl alcohol solutions*

SP. GR. 15.56° 15.56°	SCALE READINGS		DIFFER- ENCES	SP. GR. 15.56° 15.56°	SCALE READINGS		DIFFER- ENCES
	ETHYL ALCOHOL	METHYL ALCOHOL			ETHYL ALCOHOL	METHYL ALCOHOL	
1.0000	15.0	15.0	0.0	.9720	51.5	27.0	24.5
.9990	15.8	15.3	0.5	.9710	53.0	27.5	25.5
.9980	16.6	15.6	1.0	.9700	54.6	28.1	26.5
.9970	17.5	15.9	1.6	.9690	56.1	28.7	27.4
.9960	18.5	16.2	2.3	.9680	57.6	29.2	28.4
.9950	19.4	16.5	2.9	.9670	59.1	29.6	29.5
.9940	20.4	16.9	3.5	.9660	60.6	30.1	30.5
.9930	21.4	17.2	4.2	.9650	62.0	30.6	31.4
.9920	22.5	17.5	5.0	.9640	63.3	31.0	32.3
.9910	23.6	17.9	5.7	.9630	64.6	31.5	33.1
.9900	24.7	18.2	6.5	.9620	65.8	31.9	33.9
.9890	25.9	18.6	7.3	.9610	67.0	32.4	34.6
.9880	27.1	19.0	8.1	.9600	68.1	32.8	35.3
.9870	28.4	19.5	8.9	.9590	69.2	33.3	35.9
.9860	29.6	19.9	9.7	.9580	70.2	33.7	36.5
.9850	31.0	20.4	10.6	.9570	71.2	34.1	37.1
.9840	32.4	20.8	11.6	.9560	72.1	34.5	37.6
.9830	33.8	21.3	12.5	.9550	73.0	34.9	38.1
.9820	35.2	21.8	13.4	.9540	73.8	35.3	38.5
.9810	36.7	22.3	14.4	.9530	74.6	35.6	39.0
.9800	38.3	22.8	15.5	.9520	75.4	35.9	39.5
.9790	39.9	23.4	16.5	.9510	76.2	36.2	40.0
.9780	41.5	24.0	17.5	.9500	76.9	36.5	40.4
.9770	43.1	24.5	18.6	.9490	77.6	36.8	40.8
.9760	44.8	25.0	19.8	.9480	78.3	37.0	41.3
.9750	46.5	25.5	21.0	.9470	79.0	37.3	41.7
.9740	48.2	26.0	22.2	.9460	79.7	37.6	42.1
.9730	49.8	26.5	23.3				

The scale readings are applicable only to instruments calibrated in the arbitrary scale units proposed by Pulfrich, *Z. angew. Chem.*, 1899, p. 1168. According to this scale,  $14.5 = 1.33300$ ,  $50.0 = 1.34650$ , and  $100.0 = 1.36464$ . If the instrument used is calibrated in other arbitrary units, the refractive index corresponding to the observed reading can be converted into the equivalent Zeiss reading by referring to XLIII, Table 20.

#### *Tetramethylammonium Iodide Method<sup>5</sup>—Tentative*

28

#### PREPARATION OF SAMPLE

Transfer to distilling flask a quantity of sample that contains 20–25 ml of absolute alcohol and distil slowly, collecting distillate in 50 ml volumetric flask. When nearly to mark, disconnect receiver and adjust to mark at room temp. with  $H_2O$ .

29

#### APPARATUS (SEE FIG. 21)

Connect by stopcock (C), reaction flask (A) with bulb holding ca 50 ml and side inlet tube (B) to reservoir (D) of ca 25 ml capacity. Reaction flask also has second side tube (E) thru which  $CO_2$  is conducted to help carry over iodides into receiving flask (F) and thru condenser (G), which is surrounded by 12" water jacket (H). Maintain the  $H_2O$  in jacket at 50–55° by means of  $H_2O$  in flask (I) heated by burner (J). Surround reaction flask by bath (K), which contains ice and  $H_2O$  at

beginning of operation and is later heated by a flame and maintained at 75–80° during remainder of determination. By means of ground-glass joint connect outlet tube of condenser to tube M, which extends to bottom of receiving flask thru one hole of 2-holed rubber stopper. Have tube N pass thru second hole of same stopper and from thence to empty 50 ml Erlenmeyer flask (O), from which a second tube (P) passes below surface of the dilute  $\text{H}_2\text{SO}_4$  contained in second 50 ml Erlenmeyer flask (Q), which is also fitted with outlet tube (R) leading to surrounding atmosphere. Convey overflow from condenser to beaker (S) thru tube (T), passing thru stopper (U), which also holds thermometer (V). Keep receiving flask (125 ml Erlenmeyer) cold by immersing in bath (W) containing ice and  $\text{H}_2\text{O}$ .

To collect precipitate, use sintered glass filtering crucibles similar to Jena No. 1 G 4.

## 30

## REAGENTS

(a) *Trimethylamine soln.*—Cool 100 g of anhydrous trimethylamine in sealed container below boiling point (+3°) with ice and salt or by placing overnight in cold room maintained at temp. below freezing. Similarly, cool ca 1 liter of absolute alcohol and a 1 liter graduated flask. Transfer ca 700 ml of the absolute alcohol to flask, open container of the trimethylamine, and transfer the liquid to flask, washing out vessel with small portions of the cold absolute alcohol. Fill flask to within 50–75 ml of mark with the alcohol, mix, warm gradually to temp. of laboratory, fill to mark with absolute alcohol, and mix thoroly.

(b) *Wash soln.*—Place ca 0.25 g of tetramethylammonium iodide, obtained from a determination of methoxyl, in 500 ml flask, fill to convenient height with absolute alcohol, stopper, and shake to saturate liquid with the salt. Filter soln as needed thru white ribbon filter paper.

(c) *Carbon dioxide.*—Obtain from tank fitted with reducing valve and rubber tube connected to reaction flask.

## 31

## DETERMINATION

Raise temp. of the  $\text{H}_2\text{O}$  in condenser jacket (H) to 50–55° by means of flame under flask (I). Place 15 g of I and 2 g of red phosphorus in reaction flask A, attach flask to apparatus, and surround with bath of ice and  $\text{H}_2\text{O}$  (K). Introduce 2.5 ml of alcohol into reaction flask thru reservoir (D). Measure into reservoir 10–20 ml of sample, which should contain not more than 0.160 g of methyl alcohol nor more than 7 ml of ethyl alcohol. Place 25 ml of wash soln in receiving flask (F), connect flask to apparatus by means of tube M, and surround it with the bath containing ice and  $\text{H}_2\text{O}$  (W). Attach  $\text{CO}_2$  tank and so adjust as to make it possible to start current of gas at moment's notice. Stir ice and  $\text{H}_2\text{O}$  in the bath to cool reaction flask to as near 0° as possible. Adjust stopcock (C) so that sample will flow slowly down cold sides of reaction flask (A). (Addition of 10 ml of sample should require 3–5 min.) Stir ice and  $\text{H}_2\text{O}$  constantly during addition. When all the sample has run in wash sides of reservoir with two or three small portions of  $\text{H}_2\text{O}$ , using 5–10 ml, and add washings to contents of reaction flask. Fill reservoir with  $\text{H}_2\text{O}$  to prevent leakage. Remove ice from bath, leaving the cold  $\text{H}_2\text{O}$ . (The mixture in reaction flask should now consist of two layers: bottom one, dark red; upper one, colorless or nearly so. The layers will gradually mingle. If vapors of HI are seen to rise from surface of liquid, due to violence of reaction during mixing of the two layers, cool by stirring bath, adding a small piece of ice if necessary to prevent mixture from boiling or giving off acid vapors too rapidly.)

When contents of reaction flask have become homogeneous except for the floating particles of phosphorus, place burner under bath and heat fairly rapidly to 75°.

During heating add to receiving flask 10 ml of trimethylamine soln and 25 ml of wash soln, and attach flask containing the dilute  $\text{H}_2\text{SO}_4$  to prevent the trimethylamine from escaping into the air. When contents of reaction flask begin to boil, turn on the  $\text{CO}_2$  at rate of ca 50 bubbles per min., counting them as they rise from tube in receiving flask. Allow distillation to proceed 1.5–2 hours, maintaining temp. of bath at  $75\text{--}80^\circ$ , jacket (H) at  $50\text{--}55^\circ$ , and bath (W) at or near  $0^\circ$ .

Disconnect receiving flask and wash out tube (M) with 10–15 ml of wash soln, using rubber policeman to scrub off any crystals that may adhere to outside of tube and small glass rod to remove crystals from inside, if necessary. Stopper flask and let stand at room temp. overnight. (Stopper should remain loose until contents of flask reach temp. of room.) Filter mixture on weighed sintered glass crucible, using 35–40 ml of wash soln to transfer precipitate from flask to crucible. If filtrate becomes cloudy or crystals separate out, do not be concerned since the soln may contain large quantities of trimethylethylammonium iodide, which is only soluble to extent of ca 4 g per 100 ml of absolute alcohol, and large quantities of crystals are deposited due to rapid evaporation of alcohol in suction flask. At this point wash off outside of crucible with 95% alcohol to remove the crystals of trimethylethylammonium iodide that have formed on bottom and lower sides of crucible, and suck dry. Now wash contents and inside of crucible three times in following manner: Turn off suction, add ca 5 ml of wash soln by pouring down sides of crucible, and mix liquid with crystals by rotating crucible or by stirring contents with small glass rod or fine stream of wash soln from wash bottle. Wash off rod. Cover crucible with small watch-glass and let stand 2–3 min. Suck dry. After third washing, remove crucible from holder, and carefully wash off outside of crucible with 95% alcohol, sucking dry at once if any liquid gets on bottom of sinter. Dry at  $100\text{--}110^\circ$  1 hour, cool in desiccator, and weigh. Weight of precipitate  $\times 0.15933$  = weight of methyl alcohol in portion taken for analysis.

In determining 5 mg or less, Pregl filtering tubes may be used to advantage.

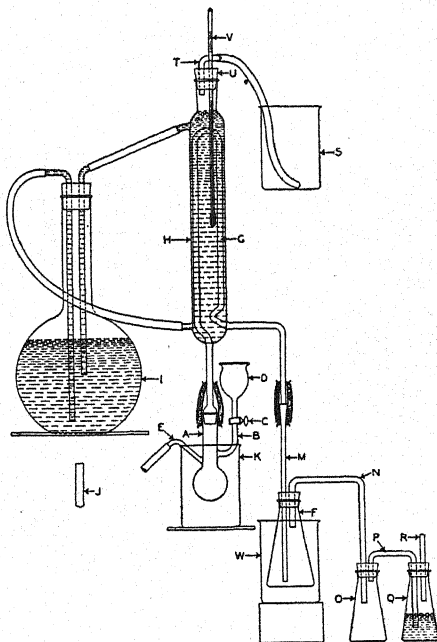


FIG. 21.—APPARATUS FOR DETERMINATION OF METHYL ALCOHOL

32

COLORING MATTERS—TENTATIVE.—See XXI.

33

WATER-INSOLUBLE COLOR—TENTATIVE

Evaporate 50 ml of sample just to dryness on steam bath. Take up with ca 15 ml of cold  $\text{H}_2\text{O}$ , filter, and wash until filtrate amounts to nearly 25 ml. To this filtrate add 25 ml of absolute alcohol, or 26.3 ml of alcohol, and make up to 50 ml with  $\text{H}_2\text{O}$ . Mix thoroly and compare in colorimeter with original material. Calculate from these readings percentage of color insoluble in  $\text{H}_2\text{O}$ .

34

COLOR INSOLUBLE IN AMYL ALCOHOL<sup>a</sup>—TENTATIVE

Evaporate 50 ml of sample just to dryness on steam bath. Dissolve residue in H<sub>2</sub>O and 95% alcohol and make to volume of 50 ml, using total volume of 26.3 ml of 95% alcohol. Place 25 ml of this soln in separatory funnel and add 20 ml of freshly shaken Marsh reagent (100 ml of pure amyl alcohol, 3 ml of sirupy H<sub>3</sub>PO<sub>4</sub>, and 3 ml of H<sub>2</sub>O), shaking lightly so as not to form an emulsion. Allow layers to separate and repeat this shaking and standing twice. After layers have separated completely draw off lower or aqueous layer, which contains the caramel, into 25 ml cylinder and make up to volume with alcohol, 50% by volume. Compare this soln in colorimeter with the untreated 25 ml. Calculate from this reading percentage of color insoluble in amyl alcohol.

## ARTIFICIAL COLORS

35

*Marsh Test<sup>7</sup>—Tentative*

To 10 ml of sample in 20 ml test tube, add sufficient Marsh reagent, 34, to nearly fill tube, and shake several times. Allow layers to separate. Color in lower layer indicates that sample has been colored with caramel, a coal tar dye, or with extractive material from uncharred white oak chips.

In absence of any color, test 10 ml in same manner, using sufficient fusel oil, amyl alcohol, or pentasol to nearly fill tube, and shaking several times. A deeply colored lower layer indicates a coal tar dye. Ascertain its identity as directed under XXI. To confirm caramel apply the following modified Marsh test.

36

*Modified Marsh Test<sup>8</sup>—Official*

Place 25 ml of the spirits in 150 ml beaker marked to show volumes of 13 ml and of 25 ml; add 0.5 ml of glacial acetic acid, 0.75 g of Zn acetate crystals, U.S.P., and mix. When crystals are nearly dissolved, boil down rapidly over flame to 13 ml mark, stirring frequently to prevent bumping or spattering. If liquid should inadvertently go below 13 ml mark, fill to that mark with H<sub>2</sub>O, and set aside to cool. When cooled to room temp. fill to 25 ml mark with 95% alcohol, mix, and allow to stand 2–3 min. Mix again, and filter thru double filter (folded or S. & S. white ribbon). Mix filtrate and transfer 6 ml to 6" test tube; add 12 ml of Marsh reagent, 34, and mix thoroly until the voluminous white precipitate that forms when liquids first mix goes back into soln. Allow to stand until layers separate, then pour off 4 ml of upper layer into graduated cylinder and in its place in test tube pour 4 ml of 88% grade ethyl acetate; mix, and allow to stand until layers separate. A dark brown color in lower layer indicates that caramel is present. If, however, lower layer is colorless and a positive Marsh test was obtained under 35, coloring from uncharred white oak chips is indicated. If lower layer has reddish shade, coal tar colors may be present. Confirm presence of coal tar color by transferring some of remaining filtrate to porcelain dish and adding a few drops of HCl. If coal tar colors are present, soln may become red. For further confirmation add SnCl<sub>2</sub> soln, which will decolorize the soln. Use clear light of open window as background for examining colors obtained in these tests.

## CORDIALS AND LIQUEURS—TENTATIVE\*

37

## PHYSICAL EXAMINATION

Note and record following: (1) Appearance, whether bright or turbid and whether there is any sediment; (2) color and depth of color; (3) odor; (4) taste.

\* Unless otherwise indicated.

38

SPECIFIC GRAVITY.—See XIV, 3.

39

## ALCOHOL

(a) *By weight*.—Proceed as directed under 3.(b) *By volume*.—Proceed as directed under 4 or 5.

## METHYL ALCOHOL

40

## PREPARATION OF SAMPLE

Measure into distilling flask such a quantity of sample as contains 20–25 ml of absolute alcohol, add sufficient  $H_2O$  to make total volume ca 100 ml, and distil, collecting ca 50 ml of distillate. To distillate add 4 g of NaCl for each 10 ml of  $H_2O$ , and allow to stand several hours to reach saturation point. Transfer to separatory funnel, using ca 10 ml of saturated NaCl soln to wash out container, and shake with 25 ml of petroleum benzin. When separation is complete, transfer the  $H_2O$  soln to second separatory funnel containing 25 ml of petroleum benzin; shake, and transfer the  $H_2O$  soln to third separatory funnel, also containing 25 ml of petroleum benzin; shake and when separation is complete, drain off  $H_2O$  soln into 200 ml distilling flask. In meantime, add to first funnel 25 ml of saturated NaCl soln and follow sample thru with this soln, finally adding washings to sample soln in distilling flask. Repeat this operation with a second 25 ml portion of saturated salt soln, finally adding this also to distilling flask. Distil mixture into 50 ml volumetric flask, using suitable adapter. When 48–49 ml has distilled over, disconnect apparatus and fill flask to mark with  $H_2O$ . Mix, and determine methyl alcohol as directed under 31.

41

## ALDEHYDES

Measure 100–200 ml of sample into distillation flask. If solid content is 25 g per 100 ml or less, add 12.5–25 ml of  $H_2O$ ; if greater than 25 g per 100 ml, add 5 ml of  $H_2O$  for each 10 g of solid matter present, and distil slowly, collecting volume of distillate equal to that of the sample, and proceed as directed under 13.

42

## FURFURAL

Treat a portion of prepared distillate, 41, as directed under 17.

43

## FUSEL OIL

Treat 50 ml of prepared distillate, 41, as directed under 21.

44

## TOTAL SOLIDS

(a) *From sp. gr. of dealcoholized sample—tentative*.—See XIV, 6.(b) *By evaporation—tentative*.—See XXXIV, 5.

(c) *From refractive index of dealcoholized sample—official, first action*.—Restore residue from alcohol determination to original volume by making necessary evaporation or dilution. Determine refractometer reading of the soln at 20° and obtain corresponding percentage of dry substance. From Table 3, XLIII, ascertain sp. gr. corresponding to percentage of dry substance found and multiply by percentage dry substance to obtain grams of total solids per 100 ml of sample. To obtain percentage of total solids in sample, divide grams of total solids per 100 ml by sp. gr. of sample, XIV, 3.

45

## GLYCEROL

(a) *Products containing 5 g per 100 ml or less of total solids*.—See XV, 5 or 6.(b) *Products containing more than 5 g per 100 ml of total solids*.—Measure into



porcelain dish such a quantity of sample (not to exceed 100 ml) as contains 25 g or less of solid matter and evaporate on steam bath to remove alcohol. Transfer to 500 ml Erlenmeyer flask, using such a quantity of  $H_2O$  that final volume will be ca 100 ml, and proceed as directed in **XV**, 7.

#### SUCROSE

46

##### *Method I. By Polarization*

Pipet into evaporating dish volume of sample equivalent to 52 g, as calculated from sp. gr., **XIV**, 3; exactly neutralize with normal NaOH, calculating quantity required from determination of acidity, 55; evaporate on steam bath to remove alcohol; transfer to 200 ml flask; and proceed as directed under **XXXIV**, 23 or 24, beginning "add necessary clarifying reagent, etc."

47

##### *Method II. By Reducing Sugars before and after Inversion*

Approximate sugar content of sample from total solids, 44, and pipet into porcelain dish such a quantity of sample as will contain 5–7 g of sugars; exactly neutralize with standard NaOH soln, calculating quantity required from acidity, and evaporate on steam bath to remove alcohol. Transfer to 200 ml volumetric flask, clarify with neutral Pb acetate soln, remove excess Pb with K oxalate, and proceed as directed under **XXXIV**, 29, using method given under **XXXIV**, 38, for determination of reducing sugars.

48

#### ASH

Proceed as directed under **XXXIV**, 9 or 10, using 25 ml of sample.

49

#### SOLUBLE AND INSOLUBLE ASH

Using ash obtained under 48, proceed as directed under **XXXIV**, 13.

50

#### ALKALINITY OF SOLUBLE ASH

Using soluble ash obtained under 49, proceed as directed under **XXXIV**, 14.

51

#### ALKALINITY OF INSOLUBLE ASH

Using insoluble ash obtained under 49, proceed as directed under **XXXIV**, 15.

52

#### PHOSPHORIC ACID

Evaporate 25 ml of sample to sirupy consistency on steam bath; add 7.5 ml of  $Mg(NO_3)_2$  soln, **II**, 7(e); mix thoroly, continue evaporation as far as possible on steam bath, and proceed as directed under **XII**, 28, beginning "heat on electric hot plate ( $180^\circ$ ). Determine  $P_2O_5$  on soln obtained as directed under **II**, 9 or 12.

53

#### CARAMEL.—See 35.

54

#### COAL TAR COLORS.—See XXI.

55

#### TOTAL ACIDITY

Place ca 600 ml of  $H_2O$  in 800 ml beaker, add ca 1 ml of phenolphthalein indicator, and titrate to pink color with 0.1 *N* NaOH. Add 10–20 ml of sample (unless this quantity gives soln such a deep color that it will obscure end point, in which case 5 ml may be used) and titrate to pink color comparable to that of the soln before sample was added. Calculate acidity as g per 100 ml of sample in terms of predominating acid present in the sample.

56

## PRELIMINARY PROCEDURE FOR CHARACTERISTIC ACIDS

Measure out such a volume of sample as contains not more than 30 g of solid matter and not more than 200 mg of the acid to be determined, as calculated from acidity; evaporate to ca 30 ml, add 6 ml of 1 *N* NaOH, and let stand at least 3 hours. Add 8 ml of 1 *N* H<sub>2</sub>SO<sub>4</sub>, transfer to 250 ml volumetric flask, using 10 ml of H<sub>2</sub>O and sufficient alcohol to fill flask to mark; mix and let stand 15 min. Filter thru thin layer of absorbent cotton, protecting liquid against evaporation. Transfer 200 ml of filtrate to centrifuge bottle and proceed with determination of the acid as directed.

57

## TARTARIC ACID

Using material in centrifuge bottle, proceed as directed under XXVI, 27 or 29.

58

## CITRIC ACID

Using material in centrifuge bottle, proceed as directed under XXVI, 31.

59

## MALIC ACID

Using material in centrifuge bottle, proceed as directed under XXVI, 34.

60

## VOLATILE ESTERS—OFFICIAL, FIRST ACTION

Measure 100–500 ml of sample into distilling flask and steam distil as directed under XIII, 26, collecting volume of distillate at least twice as great as volume of alcohol contained in sample. (If determination 61 is to be made, use 500 ml sample.) Disconnect apparatus and wash out condenser with a little H<sub>2</sub>O. Add ca 1 ml of phenolphthalein indicator and titrate to pink color that persists at least 1 min., using 0.1 *N* NaOH or KOH. Add to soln a measured excess of 25–50 ml of 0.1 *N* alkali, reflux 1 hour, cool, and titrate excess of alkali with 0.1 *N* H<sub>2</sub>SO<sub>4</sub>. Calculate number of ml of 0.1 *N* alkali used in saponification of esters as ethyl acetate. 1 ml of 0.1 *N* alkali = 8.8 mg of CH<sub>3</sub>COOC<sub>2</sub>H<sub>5</sub>.

61

GAMMA UNDECALACTONE (QUALITATIVE)<sup>a</sup>—OFFICIAL, FIRST ACTION

(Peach and Apricot Cordials.)

Make distinctly alkaline the soln obtained under 60 and evaporate to dryness on steam bath. Take up residue in ca 25 ml of H<sub>2</sub>O, transfer to separatory funnel, acidify with H<sub>2</sub>SO<sub>4</sub> (1+1), let stand 10 min. to permit lactones to form, and extract 3 times with ca 20 ml of ether. Unite ether extracts and wash 3 times by shaking with 10 ml portions of normal Na<sub>2</sub>CO<sub>3</sub> soln. Permit ether soln to evaporate spontaneously in small beaker. To residue add a few drops of N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O soln (42% in H<sub>2</sub>O) and mix thoroly; if white solid matter separates out in a few minutes, gamma undecalactone is present. Allow mixture to stand 15–20 min., place on steam bath, and heat until ammoniacal odor is no longer evident. Add 1 ml of normal butyl alcohol and warm until clear soln is obtained, adding a few additional drops of the alcohol if necessary to dissolve residue completely. Remove from steam bath and permit butyl alcohol to evaporate spontaneously. (This usually occurs overnight, but longer time may be necessary if much butyl alcohol has been used.) Examine colorless or slightly yellowish crystals under microscope. (Hydrazino-γ-undecalactone has a characteristic odor similar to that of the lactone itself.)

62

## OPTICAL PROPERTIES OF HYDRAZINO-γ-UNDECALACTONE

In ordinary light the substance is seen to consist of lath-like rods, many of them

more or less split at ends. In parallel polarized light (crossed nicols), substance is characterized by not extinguishing sharply, most of rods remaining essentially bright when stage is rotated. Occasionally there are found crystals that extinguish sharply, have square ends, and show straight extinction and negative elongation. In convergent polarized light (crossed nicols) partial biaxial interference figures, usually showing one optic axis up or slightly inclined to normal, are of frequent occurrence. The refractive indices, as determined by immersion method, are as follows:  $\alpha = 1.483$  (not common);  $\beta = 1.525$  (most frequently occurring of indices and shown lengthwise on rods);  $\gamma = 1.555$  (occurring crosswise on rods which show straight extinction and negative elongation); all  $\pm 0.003$ .

#### BENZALDEHYDE<sup>10</sup>—OFFICIAL, FIRST ACTION

63

##### REAGENT

*Phenylhydrazine soln.*—Add 1.5 ml of glacial acetic acid and 1 ml of newly distilled phenylhydrazine to 20 ml of  $H_2O$  and filter thru moistened double white ribbon filter.

64

##### DETERMINATION

Measure into distilling flask such a quantity of sample as contains 30 ml of absolute alcohol, dilute to such a volume that mixture will contain 300 ml of  $H_2O$  in addition to that required to dissolve sugar present (1 g of sugar requires 0.5 ml of  $H_2O$ ), and distil off 300 ml into 500 ml Erlenmeyer flask. Add 10 ml of the reagent and shake 5 min. Filter on Gooch with thin mat, and wash with  $H_2O$  and finally with two 10 ml portions of 10% alcohol. Dry in vacuum desiccator over  $H_2SO_4$  24 hours, excluding light, or at  $70^\circ$  under 100 mm or less of pressure 2 hours. Wt. of precipitate  $\times 0.5408$  = benzaldehyde.

65

##### THUJONE<sup>11</sup>—TENTATIVE

To 500 ml of absinth add 1 ml of freshly distilled aniline and 1 ml of sirupy phosphoric acid, and reflux 30 min. on steam bath. Distil off two 100 ml portions, reject first, and test second for thujone as follows:

Add 0.5 g of semicarbazide hydrochloride and 0.6 g of anhydrous Na acetate (or 1.0 g of the crystallized salt) and allow mixture to stand overnight. Distil off alcohol at as low a pressure as possible. Steam distil to remove essential oils and other volatile material, collect, and reject ca 15 ml of distillate. Wash down condenser with a little alcohol and with  $H_2O$ . Cool sample, add 1 ml of  $H_2SO_4$  (1+1), and again steam distil, this time collecting 20 ml of distillate in cylinder. Pour distillate into small separatory funnel, and add 20 ml of ether, using receiver as the measure. Shake and separate the ether solution. Add 10 ml of 65% alcohol and allow ether to evaporate spontaneously. When all ether has evaporated, note odor of residue. The odor of thujone will be apparent if 2 mg or more is present in soln, provided it is not masked by presence of other odoriferous substances. Make modified Legal test as follows:

To soln obtained as directed above, add 1 ml of 10%  $ZnSO_4$  soln and 0.25 ml of freshly prepared Na nitroprusside soln (0.1 g per ml of  $H_2O$ ). Slowly, with constant stirring, add 2 ml of 5% NaOH soln. Allow to stand 1–2 min. Add 1.5 ml of glacial acetic acid and mix. A precipitate of raspberry red color (resembling alcohol precipitate of red fruit juice) shows presence of thujone. A negative test is shown by a similar precipitate having an appearance similar to that of the alcohol precipitate from apple jelly or other light colored fruit.

## SELECTED REFERENCES

- <sup>1</sup> Vasey, Guide to the Analysis of Potable Spirits, 1904, p. 31.
- <sup>2</sup> J. Assoc. Official Agr. Chem., 22, 73, 222 (1939).
- <sup>3</sup> J. Am. Chem. Soc., 27, 964 (1905); Ind. Eng. Chem., 19, 844 (1927).
- <sup>4</sup> J. Assoc. Official Agr. Chem., 22, 154 (1939).
- <sup>5</sup> Ibid., 18, 477 (1935); 19, 82 (1936).
- <sup>6</sup> U. S. Dept. Agr. Bur. Chem. Bull., 152, p. 149.
- <sup>7</sup> J. Assoc. Official Agr. Chem., 18, 75 (1935); 19, 82 (1936).
- <sup>8</sup> J. Ind. Eng. Chem., 18, 304 (1926).
- <sup>9</sup> J. Assoc. Official Agr. Chem., 16, 420 (1933); 19, 75, 183 (1936).
- <sup>10</sup> Ibid., 182.
- <sup>11</sup> Ibid., 120; 20, 69 (1937); Schweiz. Wochschr., 49, 337, 507 (1911); Ann. chim. anal., 13, 227 (1908).

## XVII. BAKING POWDERS AND BAKING CHEMICALS

1

### PREPARATION OF SAMPLE—OFFICIAL

Remove entire sample from package, pass thru 20-mesh sieve, and mix thoroly.

### TOTAL CARBON DIOXIDE

#### *Gravimetric Method with Knorr's Apparatus<sup>1</sup>—Official*

2

### APPARATUS

Connect flask by means of ground-glass joint with glass connection thru upper part of which passes a dropping funnel, and join at side with Liebig condenser. Connect mouth of funnel by means of perforated stopper with soda lime tube. Connect upper end of condenser by rubber joint with train of absorption bulbs, the first containing  $\text{H}_2\text{SO}_4$  for drying the gas passing into next bulb, which contains 33% KOH soln. Connect to third bulb containing  $\text{H}_2\text{SO}_4$  for absorption of moisture escaping from KOH bulb, then to fourth bulb, also containing  $\text{H}_2\text{SO}_4$ , as precaution to prevent moisture from air being absorbed by train. Connect last bulb to aspirator. (Many analysts prefer to replace last bulb by two U-tubes filled with sifted soda lime.)

3

### DETERMINATION

Place 0.5–2 g of sample, quantity depending upon percentage of  $\text{CO}_2$  present, in flask, which must be perfectly dry. Close flask with stopper which carries funnel tube and tube connecting with absorption apparatus. Weigh separately second and third absorption bulbs and attach them to apparatus. If two soda lime tubes are used, weigh separately and refill first when second increases materially in weight. Nearly fill funnel tube with  $\text{H}_2\text{SO}_4$  (1+5) and place soda lime tube in position. Aspirate air thru absorption bulbs at rate of ca 2 bubbles per second. Open stopper of funnel and allow acid to run slowly into flask, taking care that evolution of gas is so gradual as not materially to increase current thru bulbs. After all acid has been introduced, close stopcock, continue aspiration, and gradually heat contents of flask to boiling. (While flask is being heated aspirator tube may be removed, altho when using ground-glass joints many analysts prefer to aspirate during entire operation.) Continue boiling a few minutes after the  $\text{H}_2\text{O}$  has begun to condense; remove flame, open stopcock, and continue aspiration while apparatus cools. Remove second and third bulbs and weigh. Increase in weight is due to  $\text{CO}_2$ .

#### *Gasometric Method<sup>2</sup> with Chittick's Apparatus—Official*

4

### REAGENTS

*Displacement soln.*—Dissolve 100 g of NaCl or  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  in 350 ml of  $\text{H}_2\text{O}$ . Add ca 1 g of  $\text{NaHCO}_3$  and 2 ml of methyl orange indicator, VI, 3(f), and then sufficient  $\text{H}_2\text{SO}_4$  (1+5) to make just acid (decided pink color). Stir until all  $\text{CO}_2$  is removed. This soln is used in the gas-measuring tube and leveling bulb and seldom needs to be replaced.

5

### APPARATUS

Connect decomposition flask (A) by means of glass T-tube (B), provided with stopcock (C), to graduated gas-measuring tube (D), which in turn is connected with leveling bulb (E). For A always use 250 ml wide-mouthed extraction flask of Pyrex

or other resistant glass fitted with two-holed rubber stopper, thru one hole of which passes extended tip of 25 ml buret (*F*) and thru other glass tube of same diameter as connecting T-tube. Use buret graduated in ml at 20°, numbered at 5 ml intervals, and provided with extra long tip bent to pass thru rubber stopper. Connect the glass tube leading from decomposition flask to T-tube by means of rubber tubing to permit rotation of flask. Use gas-measuring tube graduated in ml at 20°, the zero mark being placed at point 25 ml below top marking to allow for graduating upwards from 0 to 25 ml and downward from 0 to 200 ml. By means of long rubber tube connect gas-measuring tube with leveling bulb, which has a capacity of ca 300 ml.

## 6

DETERMINATION<sup>3</sup>

Weigh 1.7 g of prepared sample, 1, into flask *A* and connect this flask with apparatus (Fig. 22). Open stopcock *C* and by means of leveling bulb *E* bring displacement soln to 10 ml graduation above zero mark. (This 10 ml is practically equal in volume to volume of acid to be used in decomposition.) Allow apparatus to stand 1–2 min. to insure that temp. and pressure within apparatus are same as those of the room. Close stopcock, lower leveling bulb somewhat to reduce pressure within apparatus, and slowly run into decomposition flask from buret *F* 10 ml of  $\text{H}_2\text{SO}_4$  (1+5). To prevent liberated  $\text{CO}_2$  from escaping thru acid buret into air, keep displacement soln in leveling bulb at all times during decomposition at lower level than that in the gas-measuring tube. Rotate and then vigorously agitate decomposition flask to secure intimate mixture of contents. Allow to stand 5 min. to secure equilibrium. Equalize pressure in measuring tube by means of leveling bulb and read volume of gas in tube. Observe temp. of air surrounding apparatus and also barometric pressure at the time and multiply number of ml of gas evolved by factor given in table for this temp. and pressure, XLIII, 24. Divide corrected reading by 10 to obtain percentage by weight of  $\text{CO}_2$  in sample.

RESIDUAL CARBON DIOXIDE<sup>4</sup>

## 7

*Gravimetric Method—Official*

Weigh 2 g of prepared sample, 1, into flask suitable for subsequent determination of  $\text{CO}_2$ ; add 20 ml of cold  $\text{H}_2\text{O}$ ; and allow to stand 20 min. Place flask in metal drying cell surrounded by boiling  $\text{H}_2\text{O}$  and heat,

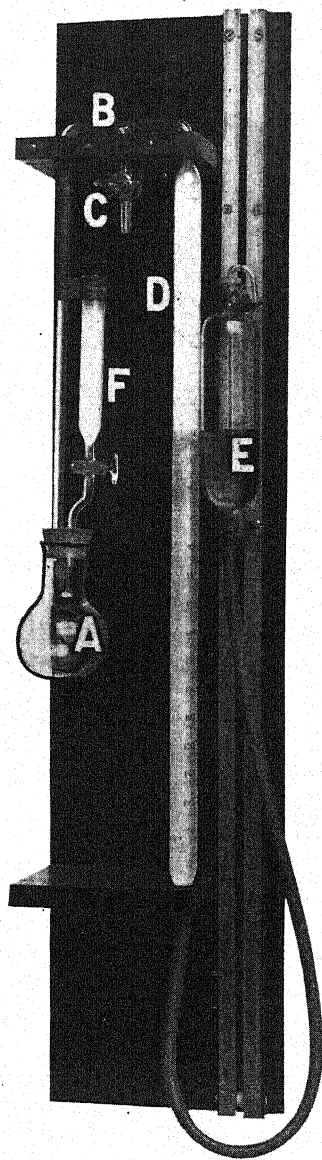


FIG. 22.—APPARATUS FOR GASOMETRIC DETERMINATION OF CARBON DIOXIDE

with occasional shaking, 20 min. To complete reaction and drive off last traces of gas from semi-solid mass, heat quickly to boiling and boil 1 min. Aspirate until air in flask is thoroly changed, and determine residual  $\text{CO}_2$  by absorption, as directed under 3.

8

*Gasometric Method<sup>s</sup>—Official*

Place 1.7 g of prepared sample, 1, in decomposition flask, A, Fig. 22; add 20 ml of  $\text{H}_2\text{O}$  and allow to stand 20 min. Place flask in metal drying cell surrounded by boiling  $\text{H}_2\text{O}$  and heat, with occasional shaking, 20 min. To complete reaction, heat quickly to boiling and boil 1 min. Cool to room temp., connect flask to apparatus described under 5, and determine  $\text{CO}_2$  present by treating with 10 ml of  $\text{H}_2\text{SO}_4$  (1+5) as directed under 6, using correction factors given in Table 24, XLIII. To prevent foaming add 1–3 drops of caprylic alcohol to baking powder in decomposition flask.

9

## AVAILABLE CARBON DIOXIDE—OFFICIAL

Subtract residual  $\text{CO}_2$ , 7, from total  $\text{CO}_2$ , 3; or subtract 8 from 6.

## NEUTRALIZING VALUE

10

*Of Acid-Reacting Materials Other Than Phosphates—Official*

Dissolve 1 g of sample in hot  $\text{H}_2\text{O}$  and titrate with 0.2 N NaOH, using phenolphthalein indicator.

11

*Of Monocalcium Phosphate<sup>s</sup>—Tentative*

(Industrial method, results approximate.)

Weigh 0.84 g of monocalcium phosphate into small beaker or casserole, add 25 ml of  $\text{H}_2\text{O}$  and 5 drops of 0.2% soln of phenolphthalein,<sup>7</sup> and titrate with 0.2 N NaOH, to faint pink. Heat to boiling, boil 1 min., and again continue titration, while soln is hot, to faint pink color, adding bulk of the standard alkali soln rapidly and with vigorous stirring. Total buret reading  $\times 2$  = neutralizing strength of 100 parts of phosphate in terms of  $\text{NaHCO}_3$ .

12

TARTARIC ACID, FREE OR COMBINED (QUALITATIVE TEST)<sup>s</sup>—TENTATIVE

(Applicable in presence of phosphates.)

Shake repeatedly ca 5 g of sample with ca 250 ml of cold  $\text{H}_2\text{O}$  in flask and allow insoluble portion to subside. Decant soln thru filter and evaporate filtrate to dryness. Powder residue, add few drops of 1% resorcinol soln, XXXIV, 96, and ca 3 ml of  $\text{H}_2\text{SO}_4$ , and heat slowly. Tartaric acid is indicated by a rose-red color, which is discharged on dilution with  $\text{H}_2\text{O}$ .

## CREAM OF TARTAR AND FREE TARTARIC ACID IN TARTRATE POWDERS—OFFICIAL

13

*Total, Combined, and Free Tartaric Acid*

To 2.5 g of baking powder in 250 ml volumetric flask, add 100 ml of  $\text{H}_2\text{O}$  at ca  $50^\circ$ , and allow to stand at room temp. ca 30 min., shaking occasionally. Cool, dilute to mark with  $\text{H}_2\text{O}$ , shake vigorously, and filter thru large fluted paper. Pipet 2 portions of 100 ml each of the clear filtrate into 250 ml beakers and evaporate to ca 20 ml. To one portion add 3.5 ml of about normal KOH. Mix well and add 2 ml of glacial acetic acid. Again mix well and add 100 ml of alcohol, stirring constantly.

Treat other portion in similar manner, but use normal NaOH instead of KOH. Cool mixtures to ca 15°, stir vigorously ca 1 min., and allow to remain in refrigerator overnight. Collect precipitate in Gooch on thin, tightly tamped pad of asbestos. Rinse beaker with ca 75 ml of ice-cold 80% alcohol, carefully washing down sides of beaker. Finally, wash sides of crucible with 25 ml of alcohol and suck dry. Transfer contents of crucible to original beaker with ca 100 ml of hot H<sub>2</sub>O and titrate with 0.1 N alkali, using phenolphthalein indicator. Designate titer of portion treated with KOH as "A" and that treated with NaOH as "B."

14

## CALCULATIONS

Percent total tartaric acid =  $1.5(A + 0.6)$ .

Percent cream of tartar =  $1.88(B + 0.6)$ .

Percent free tartaric acid =  $1.5(A - B)$ .

In above formulas "0.6" represents solubility of cream of tartar in reaction mixture in terms of 0.1 N alkali.

*Free Tartaric Acid (Direct Determination)*

15

## REAGENT

*Saturated alcohol.*—To ca 50 g of purest cream of tartar (finely powdered) in Erlenmeyer flask, add ca 100 ml of 95% alcohol and 100 ml of H<sub>2</sub>O, shake vigorously several minutes, and allow to stand 15 min., shaking occasionally. Filter on paper in Büchner funnel, and wash salt with ca 200 ml of diluted 95% alcohol (1+1), then with 95% alcohol, and finally with ether. Dry at temp. of boiling H<sub>2</sub>O. To 500 ml of *absolute* alcohol add ca 5 g of the purified cream of tartar and allow to stand 2 hours, shaking occasionally. If the cream of tartar has been properly purified a blank (50 ml of CHCl<sub>3</sub> + 150 ml of the saturated alcohol) should not require more than 0.15 ml of 1 N alkali to neutralize 100 ml of the mixture.

16

## DETERMINATION

Weigh 1.25 g of baking powder into an absolutely *dry* 200 ml volumetric flask, add 50 ml of CHCl<sub>3</sub>, and allow to stand ca 5 min., shaking occasionally. (If, upon addition of the CHCl<sub>3</sub>, the powder sticks to bottom of the flask, moisture is indicated and determination should be discarded.) Add 100 ml of the saturated alcohol, shake ca 5 min., and allow to stand 30 min., shaking at frequent intervals. (It is not necessary to filter the alcohol reagent.) Make to mark with the saturated alcohol, shake a few minutes, and filter thru large fluted paper. Titrate 100 ml of clear filtrate with 0.1 N alkali (phenolphthalein). The quantity (ml) of alkali used  $\times 1.2$  = percentage of free tartaric acid.

17

## FREE TARTARIC ACID (QUALITATIVE TEST)—OFFICIAL

Extract 5 g of sample with absolute alcohol and evaporate the alcohol from the extract. Dissolve residue in NH<sub>4</sub>OH (1+10), transfer to test tube, add good sized crystal of AgNO<sub>3</sub>, and heat gently. Tartaric acid is indicated by formation of a silver mirror. If desired, the absolute alcoholic extract may be tested as directed under 12.

## STARCH

18

*Direct Inversion Method—Official*

(For baking powders and baking chemicals free from calcium.)

Weigh 5 g of sample into 500 ml volumetric flask and proceed as directed under XXVII, 30.



19

*Indirect Method<sup>9</sup>—Official*

(For baking powders and baking chemicals containing calcium.)

Mix 5 g of sample with 200 ml of HCl (1+11) in 500 ml volumetric flask and allow mixture to stand an hour, shaking frequently. Filter on 11 cm hardened filter, taking care to obtain clear filtrate. Rinse flask once without attempting to remove all the starch, and wash paper twice with cold H<sub>2</sub>O. Carefully wash starch from paper back into flask with 200 ml of H<sub>2</sub>O. Add 20 ml of HCl (sp. gr. 1.125) and proceed as directed under XXVII, 30. (Treatment with the HCl, without dissolving the starch, removes effectively the Ca, which otherwise would be precipitated as tartrate by the alkaline Cu soln.)

20

*Modified McGill Method—Tentative*

Digest 1 g of sample with 150 ml of HCl (1+11) 24 hours at room temp., with occasional shaking. Filter on Gooch crucible, wash thoroly with cold H<sub>2</sub>O and then once with alcohol and once with ether. Dry at 110° (4 hours usually sufficient), cool, and weigh. Burn off starch, weigh again, and determine the starch by difference. (Results by this method on cream of tartar powders and tartaric acid powders agree closely with those obtained by Cu reduction. Results on other types of baking powders are usually satisfactory, but in some instances they may be over 2% too high.)

## ALUMINUM

*Qualitative Test<sup>10</sup>—Tentative*

(In presence of phosphates.)

21

## REAGENTS

- (a) *Hydrochloric acid soln.*—Approximately normal. Dilute 9 ml of HCl to 100 ml.
- (b) *Ammonium acetate soln.*—3 N. Dissolve 23.1 g of NH<sub>4</sub> acetate in H<sub>2</sub>O and dilute to 100 ml.
- (c) *Aurintricarboxylic acid soln.*—0.1%. Dissolve 0.1 g in H<sub>2</sub>O and dilute to 100 ml.

22

## DETECTION

Dissolve 1–5 g of the baking powder in 5 ml of the HCl and 5 ml of the NH<sub>4</sub> acetate. Add 5 ml of the 0.1% soln of aurintricarboxylic acid, mix, and allow lake formation to take place. Make soln alkaline with NH<sub>4</sub>OH containing a small quantity of (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>. A bright persistent red precipitate indicates presence of Al.

*Quantitative Determination by Precipitation with Phenylhydrazine—Tentative*

23

## REAGENTS

- (a) *Ammonium bisulfite soln.*—Pass SO<sub>2</sub> into cool soln of NH<sub>4</sub>OH (1+1) until color of soln becomes distinctly yellow.
- (b) *Phenylhydrazine bisulfite soln.*—To a few ml of phenylhydrazine add gradually saturated soln of SO<sub>2</sub> until precipitate of phenylhydrazine sulfite, which at first separates out in crystals, is almost redissolved. If precipitate is completely dissolved, add a drop or two of phenylhydrazine until a slight precipitate is obtained. Filter soln before using. (From 5–10 ml of this soln in 100 ml of H<sub>2</sub>O is sufficient concentration for washing the Al(OH)<sub>3</sub> precipitate. If well stoppered, this concentrated soln of phenylhydrazine bisulfite will keep indefinitely.)

24

## DETERMINATION

Ignite 3 g of the baking powder at a temp. not exceeding 550°. As soon as the C has burned off, take up residue in HCl (4+10) and boil gently to assist soln. Filter into 300 ml volumetric flask and wash with hot H<sub>2</sub>O. Ignite insoluble residue and filter paper in Pt crucible and fuse residue with ca 2 g of Na<sub>2</sub>CO<sub>3</sub>. Dissolve fused mass in H<sub>2</sub>O and HCl and transfer to the volumetric flask containing original filtrate. Cool, and make up to volume.

Transfer 100 ml aliquots to 400 ml beakers. Heat nearly to boiling, add NH<sub>4</sub>OH (1+10) until slight permanent precipitate forms, then just redissolve this precipitate with a drop or two of the dilute HCl. Add dropwise with constant stirring 10 or 12 drops of saturated soln of NH<sub>4</sub>HSO<sub>3</sub>. Then add to hot soln sufficient phenylhydrazine to precipitate the Al(OH)<sub>3</sub> completely (1 or 2 ml; an excess colors soln yellow). If a permanent precipitate does not form at this point, add NH<sub>4</sub>OH (1+10) carefully, dropwise, just to permanent precipitate, and then complete precipitation by adding a few more drops of the phenylhydrazine. Let stand a few minutes for precipitate to settle, then filter while still warm. Wash precipitate with warm H<sub>2</sub>O containing the phenylhydrazine bisulfite until washings give no test for iron when yellow NH<sub>4</sub> sulfide is added.

Place filter paper containing precipitate in weighed Pt crucible. Dry, char, and ignite at low temp. After filter paper has completely burned, continue ignition at bright red heat (850–950°) to constant weight. Weigh quickly with cover on crucible as precipitate is very hygroscopic. A second weighing is always necessary. The precipitate consists of Al<sub>2</sub>O<sub>3</sub> and Al phosphate.

Fuse ignited precipitate with ca 2 g of Na<sub>2</sub>CO<sub>3</sub> and dissolve fusion in HNO<sub>3</sub> (1+9). Transfer to 250 ml beaker and boil to insure that all the phosphoric acid is in ortho state. Cool. Transfer to 200 ml flask, make up to volume, and use 50 ml aliquots to determine the P<sub>2</sub>O<sub>5</sub>. Multiply weight of P<sub>2</sub>O<sub>5</sub> obtained by 4 and subtract product from weight of combined precipitates obtained above. Difference = weight of Al<sub>2</sub>O<sub>3</sub> in 1 g of baking powder.

Weight Al<sub>2</sub>O<sub>3</sub> × 100 = percentage of Al<sub>2</sub>O<sub>3</sub>.

Percentage of Al<sub>2</sub>O<sub>3</sub> × 4.749 = percentage of Na<sub>2</sub>Al<sub>2</sub>(SO<sub>4</sub>)<sub>4</sub>.

If baking powder contains significant quantity of SiO<sub>2</sub>, remove by evaporating the HCl soln of the powder to dryness and dehydrating at 105° 2 hours. Add to dry mass 10 ml of HCl and 100 ml of H<sub>2</sub>O, boil, filter off SiO<sub>2</sub>, and proceed as directed above.

25

INSOLUBLE ASH AND PREPARATION OF SOLUTION<sup>12</sup>—OFFICIAL

Char 5 g of sample in Pt dish at a heat below redness (ca 500°). Boil carbonaceous mass with HCl (1+2.5), filter into 500 ml volumetric flask, and wash with hot H<sub>2</sub>O. Return residue, together with paper, to Pt dish, and burn to white ash. Boil again with the dilute HCl, filter, wash, unite two filtrates, and dilute to 500 ml. Incinerate residue after last filtration and weigh ash insoluble in acid.

26

IRON AND ALUMINUM<sup>12</sup>—OFFICIAL

Draw 100 ml aliquot of prepared soln, 25, and separate SiO<sub>2</sub> if necessary. Mix soln with 10% Na phosphate soln in excess. Add NH<sub>4</sub>OH until permanent precipitate is obtained, then HCl, dropwise, until precipitate is dissolved. Bring soln to boil and boil 2–3 min.; mix with considerable excess of 50% NH<sub>4</sub> acetate soln and 4 ml of 80% acetic acid. As soon as precipitate of Al phosphate, mixed with Fe phosphate, has settled, collect on filter, wash with hot H<sub>2</sub>O, ignite, and weigh. Fuse mixed phosphates with 10 parts of Na<sub>2</sub>CO<sub>3</sub>, dissolve in H<sub>2</sub>SO<sub>4</sub> (1+6), reduce

with Zn, and determine Fe by titration with standard permanganate soln (1 ml = 1 mg of Fe). In same soln determine phosphoric acid as directed under II, 9 or 12. To obtain weight of  $\text{Al}_2\text{O}_3$ , subtract sum of weights of  $\text{Fe}_2\text{O}_3$  and  $\text{P}_2\text{O}_5$  from weight of mixed phosphates.

27

CALCIUM<sup>12</sup>—OFFICIAL

Heat combined filtrate and washings obtained under 26 to 50° and add excess of saturated  $\text{NH}_4$  oxalate soln. Allow to stand in warm place until precipitate has settled, filter, wash precipitate with hot  $\text{H}_2\text{O}$ , dry, and ignite over Bunsen burner and finally over blast lamp. Cool in desiccator and weigh as  $\text{CaO}$ .

28

POTASSIUM AND SODIUM<sup>12</sup>—OFFICIAL

Evaporate aliquot of prepared soln, 25, nearly to dryness to remove excess of  $\text{HCl}$ , dilute, and heat to boiling. While still boiling add 10%  $\text{BaCl}_2$  soln as long as a precipitate forms and then enough saturated  $\text{Ba}(\text{OH})_2$  soln to make liquid strongly alkaline. As soon as precipitate has settled, filter and wash with hot  $\text{H}_2\text{O}$ ; heat filtrate to boiling; add sufficient  $(\text{NH}_4)_2\text{CO}_3$  [1 part  $(\text{NH}_4)_2\text{CO}_3$  in 5 of  $\text{NH}_4\text{OH}$  soln (1+12)] to precipitate all the Ba; filter, and wash with hot  $\text{H}_2\text{O}$ . Evaporate filtrate to dryness and ignite residue below redness to remove  $\text{NH}_4$  salts. Add to residue a little  $\text{H}_2\text{O}$  and a few drops of the  $(\text{NH}_4)_2\text{CO}_3$  soln. Filter into weighed Pt dish, evaporate, ignite below redness, and weigh the mixed K and Na chlorides. Digest residue with hot  $\text{H}_2\text{O}$ , filter thru a small filter, and dilute filtrate, if necessary, to provide for each decigram of  $\text{K}_2\text{O}$  at least 20 ml of liquid. Acidify with a few drops of  $\text{HCl}$  and add an excess of Pt soln, II, 40(b). Evaporate on water bath to a thick paste; treat residue repeatedly with 80% alcohol, decanting thru weighed Gooch crucible or other form of filter, transfer precipitate to filter, and wash thoroly with the 80% alcohol. Dry 30 min. at 100° and weigh. Calculate the K so found to its equivalent of  $\text{KCl}$  and subtract result from weight of the mixed chlorides to obtain weight of  $\text{NaCl}$ .

29

## PHOSPHORIC ACID—OFFICIAL

Mix 5 g of sample with a little  $\text{Mg}(\text{NO}_3)_2$  soln, II, 7(e), dry, ignite, dissolve in  $\text{HCl}$  (1+2.5), and dilute soln to definite volume. In an aliquot of the soln determine phosphoric acid as directed under II, 9 or 12.

30

SULFURIC ACID<sup>12</sup>—OFFICIAL

Boil 5 g of sample 1.5 hours with mixture of 300 ml of  $\text{H}_2\text{O}$  and 15 ml of  $\text{HCl}$ . Filter, wash filter thoroly with hot  $\text{H}_2\text{O}$ , cool combined filtrate and washings, and dilute to volume of 500 ml. Determine  $\text{H}_2\text{SO}_4$  in an aliquot of 100 ml as directed under XII, 27.

31

## AMMONIA—OFFICIAL

Introduce 2 g of sample into distillation flask, add 300–400 ml of  $\text{H}_2\text{O}$  and an excess of  $\text{NaOH}$  soln (1+1), connect with condenser, and distil into measured volume of standard acid. Titrate excess of acid in distillate with standard alkali, using methyl red or cochineal indicator.

32

## ARSENIC—TENTATIVE

Introduce 5 g of sample directly into generator described under XXIX, 5; add 10 ml of  $\text{H}_2\text{O}$ , a little at a time to prevent foaming over, and then 15 ml of As-free  $\text{HCl}$ , introducing it dropwise until foaming ceases. Heat on steam bath until a drop of the mixture, when diluted and treated with I soln, shows no blue color. Then

dilute to ca 30 ml with H<sub>2</sub>O and continue as directed under **XXIX, 5**, beginning "Add 5 ml of the KI reagent." Make blank and standards for comparison by use of the As-free HCl of the same concentration as that used in determination.

## SELECTED REFERENCES

- <sup>1</sup> J. Anal. Chem., **3**, 162 (1889).
- <sup>2</sup> J. Assoc. Official Agr. Chem., **6**, 453 (1923).
- <sup>3</sup> Ibid., **10**, 36 (1927).
- <sup>4</sup> Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 169; Inland Revenue Dept. Canada Bull. **68**, p. 31; Catlin, Baking Powders; A Treatise on Their Character, Methods for Determination of Their Values, etc., p. 20.
- <sup>5</sup> J. Assoc. Official Agr. Chem., **6**, 454 (1923).
- <sup>6</sup> Ibid., **8**, 91 (1924).
- <sup>7</sup> Analyst, **47**, 339 (1922).
- <sup>8</sup> Ann. chim. anal., **4**, 263 (1899).
- <sup>9</sup> Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 174.
- <sup>10</sup> J. Am. Chem. Soc., **47**, 142 (1925).
- <sup>11</sup> J. Assoc. Official Agr. Chem., **12**, 46 (1929).
- <sup>12</sup> Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 178.
- <sup>13</sup> U. S. Dept. Agr. Bur. Chem. Bull. **13** (V), p. 596; Conn. Agr. Exp. Sta. Rpt. 1900 (II), p. 179.

## **XVIII. COFFEE AND TEA**

### **GREEN COFFEE**

#### **1                   MACROSCOPIC EXAMINATION—TENTATIVE**

A macroscopic examination usually shows presence of excessive quantities of black and blighted coffee beans, coffee hulls, stones, and other foreign matter. Separate these by hand picking and determine quantity gravimetrically.

#### **2                   COLORING MATTERS—TENTATIVE**

Shake vigorously 100 g or more of sample with cold  $H_2O$  or alcohol, 70% by volume. Strain thru coarse sieve and allow to settle. Identify soluble colors in soln and insoluble pigments in sediment as directed under **XXI**.

### **ROASTED COFFEE**

#### **3                   MACROSCOPIC EXAMINATION—TENTATIVE**

Artificial coffee beans are apparent from their regularity of form, and roasted legumes and lumps of chicory in whole roasted coffee can be picked out and identified microscopically. For ground coffee sprinkle some of sample on cold  $H_2O$  and stir lightly. Fragments of pure coffee, if not overroasted, will float, while fragments of chicory, legumes, cereals, etc., will sink immediately, chicory coloring the  $H_2O$  a decided brown. In all cases identify particles that sink by microscopical examination.

#### **4                   PREPARATION OF SAMPLE—OFFICIAL**

Grind sample to pass thru 30-mesh sieve and preserve in tightly stoppered bottle.

#### **5                   MOISTURE—TENTATIVE**

Dry 5 g of sample at temp. of boiling  $H_2O$  under pressure not to exceed 100 mm of Hg, or at temp. of 105–110° under atmospheric pressure, for 5 hours and subsequent periods of 1 hour each until constant weight is obtained. For whole coffee, grind rapidly to coarse powder and without sifting and unnecessary exposure to air weigh portions for the determination. For ground coffee, sample directly without further grinding.

#### **6                   SOLUBLE SOLIDS—TENTATIVE**

Place 4 g of prepared sample, **4**, in 200 ml flask. Add  $H_2O$  to mark, allow mass to infuse 8 hours, with occasional shaking, and let stand 16 hours longer without shaking. Filter, and evaporate 50 ml of filtrate to dryness in flat-bottomed dish. Dry at 100°, cool, and weigh.

#### **7                   ASH—OFFICIAL**

Proceed as directed under **XXXIV**, 9 or 10, using sample prepared as directed under **4**.

#### **8                   SOLUBLE AND INSOLUBLE ASH—OFFICIAL**

Proceed as directed under **XXXIV**, 13, using ash obtained under **7**.

#### **9                   ALKALINITY OF SOLUBLE ASH—OFFICIAL**

Proceed as directed under **XXXIV**, 14, using filtrate obtained under **8**.

10

## ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under XXXIII, 5, using ash obtained as directed under 7 or water-insoluble ash obtained as directed under 8.

11

## SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under II, 9 or 12, using soln obtained under 9.

12

## INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Boil insoluble ash obtained as directed under 8 with 25 ml of HCl (1+2), filter, wash thoroly with hot H<sub>2</sub>O, and determine P<sub>2</sub>O<sub>5</sub> in combined filtrate and washings as directed under II, 9 or 12.

13

## CHLORIDES—OFFICIAL

Proceed as directed under XII, 35.

## CAFFEINE

14

*Power-Chesnut Method<sup>1</sup>—Official*

Moisten 10 g of prepared sample, 4, with alcohol; transfer to Soxhlet or similar extraction apparatus; and extract with alcohol 8 hours, exercising care to assure complete extraction. Transfer extract with aid of hot H<sub>2</sub>O to porcelain dish containing 10 g of heavy MgO in suspension in 100 ml of H<sub>2</sub>O. Evaporate slowly on steam bath with frequent stirring to dry, powdery mass. Rub residue with pestle into paste with boiling H<sub>2</sub>O and transfer with hot H<sub>2</sub>O to smooth filter, cleaning dish with policeman. Collect filtrate in liter flask marked at 250 ml and wash with boiling H<sub>2</sub>O until filtrate reaches mark. Add 20 ml of H<sub>2</sub>SO<sub>4</sub> (1+9) and boil gently 30 min. with a funnel in neck of flask. Cool, filter thru moistened double paper into separatory funnel, and wash with small portions of H<sub>2</sub>SO<sub>4</sub> (1+199). Extract with 6 successive 25 ml portions of CHCl<sub>3</sub>. Wash combined CHCl<sub>3</sub> extracts in separatory funnel with 5 ml of 1% KOH soln. Filter the CHCl<sub>3</sub> into Erlenmeyer flask. Wash the KOH soln with 2 portions of CHCl<sub>3</sub> of 10 ml each, adding them to flask, together with the CHCl<sub>3</sub> washings of filter paper. Evaporate or distil on steam bath to small volume (10–15 ml), transfer with CHCl<sub>3</sub> to weighed beaker, evaporate carefully, dry 30 min. at 100°, and weigh. Test purity of residue by determining N and multiplying by factor 3.464.

With products very low in caffeine combine caffeine residues from duplicate determinations (representing 20 g of original material) and determine N as directed in II, 21 or 22, using half the quantity of reagents specified for digestion and steaming out apparatus thoroly before distilling. Distil to small volume in distilling flask to insure removal of all ammonia. Correct for blank obtained, using same reagents and apparatus, and pure sucrose in place of caffeine.

15

*Fendler-Stüber Method (Modified)<sup>2</sup>—Tentative*

(Adapted for quick results.)

Treat 10 g of prepared sample, 4, with 10 ml of NH<sub>4</sub>OH (1+2) and 200 g of CHCl<sub>3</sub> in glass-stoppered bottle; shake continuously by machine or hand 30 min., and chill in ice bath. Pour entire contents of bottle on 24 cm folded filter, covering immediately with watch-glass. Collect filtrate with funnel resting directly in neck of flask (previously weighed with stopper) and having flask surrounded with ice. Stopper as soon as soln ceases to run from funnel in a continuous stream and weigh. Evaporate on steam bath, removing last CHCl<sub>3</sub> with current of air. Digest residue

with 80 ml of hot  $\text{H}_2\text{O}$  10 min. on steam bath, shaking frequently, and let cool. Treat soln with 1%  $\text{KMnO}_4$  soln (20 ml for roasted and 10 ml for green) and let stand 15 min. at room temp., shaking occasionally. Add 2 ml of  $\text{H}_2\text{O}_2$  soln (100 ml of 3%  $\text{H}_2\text{O}_2$ , free of acetanilid, plus 1 ml of glacial acetic acid). If liquid is still red or reddish, add the  $\text{H}_2\text{O}_2$  soln, 1 ml at a time, until excess of  $\text{KMnO}_4$  is destroyed. Place flask on steam bath 15 min. and add 0.5 ml portions of the  $\text{H}_2\text{O}_2$  soln until liquid ceases to become lighter. Cool, and filter by suction thru Gooch crucible, washing with cold  $\text{H}_2\text{O}$ . Transfer filtrate to separatory funnel and extract 6 times with 25 ml portions of  $\text{CHCl}_3$ . Evaporate combined  $\text{CHCl}_3$  extracts to small volume, transfer to weighed beaker, finish evaporation, dry at  $100^\circ$  to constant weight (30 min. is usually sufficient), and weigh residue as caffeine. Weight of caffeine  $\times 2000 \div$  weight of the  $\text{CHCl}_3$  aliquot obtained from first filtration = percentage of caffeine in the 10 g sample. Test purity of residue as directed in 14.

16

## CRUDE FIBER—OFFICIAL

Proceed as directed under XXVII, 27, using prepared sample, 4.

17

## STARCH—TENTATIVE

Extract 5 g of prepared sample, 4, on hardened filter with 5 successive 10 ml portions of ether; wash with small portions of alcohol until total of 200 ml has passed thru; and proceed as directed under XXVII, 32, beginning with second sentence.

18

SUGARS<sup>a</sup>—TENTATIVE

Weigh 10 g of prepared sample, 4, into 250 ml volumetric flask; add 1 g of powdered  $\text{NH}_4\text{NaHPO}_4$  and proceed as directed under XXVII, 28, 29. Determine reduced Cu in  $\text{Cu}_2\text{O}$  precipitate either volumetrically, XXXIV, 41, or electrolytically, XXXIV, 44.

19

## PETROLEUM BENZIN EXTRACT—OFFICIAL

Dry 2 g of prepared sample, 4, at  $100^\circ$ , extract with petroleum benzin (b.p.  $35\text{--}50^\circ$ ) 16 hours, evaporate solvent, dry residue at  $100^\circ$ , cool, and weigh.

20

## TOTAL ACIDITY—TENTATIVE

Treat 10 g of prepared sample, 4, with 75 ml of alcohol, 80% by volume, in Erlenmeyer flask; stopper; and allow to stand 16 hours, shaking occasionally. Filter, and transfer aliquot of filtrate (25 ml for green coffee, 10 ml for roasted coffee) to beaker; dilute to ca 100 ml with  $\text{H}_2\text{O}$ ; and titrate with 0.1 N alkali, using phenolphthalein indicator. Express result as number of ml of 0.1 N alkali required to neutralize acidity of 100 g of sample.

21

## VOLATILE ACIDITY—TENTATIVE

Into volatile acid apparatus, XV, 24, introduce a few glass beads and over these place 20 g of the unground sample. Add 100 ml of recently boiled  $\text{H}_2\text{O}$ , place a sufficient quantity of recently boiled  $\text{H}_2\text{O}$  in outer flask, and distil until distillate is no longer acid to litmus paper (usually 100 ml of distillate will be collected). Titrate distillate with 0.1 N alkali, using phenolphthalein indicator. Express result as number of ml of 0.1 N alkali required to neutralize acidity of 100 g of sample.

## COATING AND GLAZING SUBSTANCES

22

## SUGAR AND DEXTRIN—TENTATIVE

Introduce 100 g of whole coffee into beaker, add exactly 300 ml of  $\text{H}_2\text{O}$ , stir, and allow to stand 5 min., stirring frequently. Filter thru dry filter and add carefully to

filtrate dry Pb acetate until precipitation is complete, avoiding excess of the reagent. Filter thru dry filter and remove the Pb from filtrate by addition of a slight excess of dry, powdered K oxalate. Filter thru dry filter and determine reducing sugars as invert sugar in 50 ml of the filtrate, as directed under XXXIV, 38. Invert 75 ml aliquot of filtrate as directed under XXXIV, 24(b). Cool, nearly neutralize with NaOH soln (1+1), dilute to 100 ml, and determine reducing sugars as invert sugar in resulting soln as directed under XXXIV, 38. Measure 100 ml aliquot of filtrate into 200 ml flask, add 10 ml of HCl (sp. gr. 1.125), and hydrolyze as directed under XXVII, 30. Cool, neutralize with NaOH soln (1+1), dilute to volume, filter thru dry filter, and determine reducing sugars as invert sugar in 50 ml of filtrate as directed under XXXIV, 38. Calculate reducing sugars in each instance to percentage by weight of original coffee. Calculate sucrose from reducing sugars before and after inversion as directed under XXXIV, 29, and calculate dextrin as follows: Subtract reducing sugars after inversion from reducing sugars after hydrolysis and multiply difference by factor 0.8605 to convert result to dextrin.

In some instances presence of sucrose in the water extract may be verified by polarization. Presence of dextrin in the water extract may be verified by polarization as directed under XXXIV, 31, and by erythro dextrin test (XXXIV, 94) made on water extract previous to clarification with Pb acetate.

23

## EGG ALBUMIN AND GELATIN—TENTATIVE

Treat 100 g of whole coffee with 500 ml of H<sub>2</sub>O and allow to stand 5 min., stirring frequently. Filter, and treat separate portions of filtrate with (1) 5% soln of tannic acid, and (2) Millon's reagent (XX, 24). Boil a third portion of filtrate. In the presence of egg albumin a more or less heavy precipitate will be formed in each case. As a confirmatory test, treat aliquot of filtrate with excess of tannic acid soln; add a little salt if necessary to secure flocculation of precipitate; filter; and, without washing, introduce paper and its contents into Kjeldahl flask and determine N. By this method coffee not coated with albumin or gelatin will yield less than 10 mg of N per 100 g of sample.

24

## CHICORY INFUSION—TENTATIVE

Cover 100–150 g of whole coffee with H<sub>2</sub>O; allow to soak 2–3 min., stirring frequently; and drain aqueous washings thru coarse sieve. Wash coffee upon sieve with ca 100 ml of H<sub>2</sub>O and centrifuge combined washings. Decant clear liquid from sediment, which should then be drained almost dry on filter paper. Mount sediment in chloral hydrate soln, XXXIII, 29(c), and examine under microscope for elements of chicory.

25

## FATS AND WAXES—TENTATIVE

Treat 100–200 g of the beans with low-boiling petroleum benzin 10 min., pour off petroleum benzin, and repeat process. Filter combined extracts, evaporate, and determine index of refraction and saponification number of residue as directed under XXXI, 9 and 25.

## TEA

26

## DUST, STEMS, AND FOREIGN LEAVES—TENTATIVE

Place 1 g of the tea in 300 ml casserole, add 200 ml of boiling H<sub>2</sub>O, and allow to stand 15 min. This treatment will cause the leaves to unroll, and they will then be in condition for examination as to form and structure.<sup>5</sup> A macroscopic examination will reveal presence or absence of dust or stems. Only those stems that remain floating after the leaf is thoroly infused should be regarded as woody stems<sup>6</sup> ("floaters").



## 27 PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru 30-mesh sieve.

## 28 MOISTURE—OFFICIAL.—See XXVII, 2.

## 29 WATER EXTRACT—OFFICIAL

To 2 g of the ground sample in 500 ml volumetric flask, add 200 ml of hot  $H_2O$  and boil over low flame 1 hour, rotating occasionally. Close flask with rubber stopper thru which passes glass tube 30" long for condenser. Boil very slowly so that no steam escapes from top of air condenser. Cool, dilute to volume, mix thoroly, and filter thru dry filter paper. Transfer aliquot of 50 ml to weighed dish and evaporate to dryness on steam bath. Place in oven, heat at  $100^\circ$  1 hour, cool, and weigh.

## 30 ASH—OFFICIAL.—See XXXIV, 9 or 10.

## 31 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using ash obtained under 30.

## 32 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using filtrate obtained under 31.

## 33 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 15, using insoluble ash obtained under 31.

## 34 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under XXXIII, 5, using total ash obtained as directed under 30, or insoluble residue obtained under 31.

## 35 SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under II, 9 or 12, using soln of soluble ash obtained under 32.

## 36 INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under II, 9 or 12, using soln obtained under 33.

## 37 PETROLEUM BENZIN EXTRACT—OFFICIAL.—See 19.

## 38 PROTEIN—TENTATIVE

Determine N as directed under II, 21, 22 or 23. To obtain percentage of N present as protein, subtract percentage of N present as caffeine from percentage of total N. Multiply this result by 6.25 to obtain percentage of protein.

## 39 CRUDE FIBER—OFFICIAL.—See XXVII, 27

## CAFFEINE

40 *Power-Chesnut Method*<sup>8</sup>—Official

Proceed as directed under 14.

41 *Bailey-Andrew Method*<sup>9</sup>—Official

To 5 g of prepared sample, 27, in 500 ml volumetric flask, add 10 g of heavy  $MgO$  and 200 ml of  $H_2O$ . Boil gently over low flame 2 hours, using small-bore glass tube 30" long as condenser. Cool, dilute to volume, and filter thru dry paper. Transfer aliquot portion of 300 ml, equivalent to 3 g of original material, to Erlenmeyer flask of 1 liter capacity; add 10 ml of  $H_2SO_4$  (1+9); and boil until volume is reduced to

ca 100 ml. Filter into separatory funnel, washing flask with small portions of  $\text{H}_2\text{SO}_4$  (1+99), and shake 6 times with  $\text{CHCl}_3$ , using 25, 20, 15, 10, 10, 10 ml portions. Treat combined extracts with 5 ml of 1% soln of KOH and when liquids have completely separated draw off  $\text{CHCl}_3$  layer into suitable flask or beaker. Wash the alkaline soln in separatory funnel with 2 portions of  $\text{CHCl}_3$ , of 10 ml each, and unite washings with main bulk of extract. Evaporate or distil off the  $\text{CHCl}_3$  to small bulk, transfer to weighed flask, evaporate to dryness, and further dry in oven at  $100^\circ$  to constant weight. Test the purity of residue by determining N and multiplying by factor 3.464. This gives a value for anhydrous caffeine.

TANNIN<sup>10</sup>—TENTATIVE

42

## REAGENTS

(a) *Potassium permanganate soln.*—Prepare soln containing 1.33 g per liter and obtain its equivalent in terms of 0.1 N oxalic acid.

(b) *Indigo carmine soln.*—Prepare soln containing 6 g of indigo carmine (free from indigo blue) and 50 ml of  $\text{H}_2\text{SO}_4$  per liter.

(c) *Gelatin soln.*—Soak 25 g of gelatin 1 hour in saturated NaCl soln, heat until gelatin is dissolved, cool, and dilute with saturated NaCl soln to 1 liter.

(d) *Acid sodium chloride soln.*—Acidify 975 ml of saturated NaCl soln with 25 ml of  $\text{H}_2\text{SO}_4$ .

43

## DETERMINATION

Boil 5 g of the tea 30 min. with 400 ml of  $\text{H}_2\text{O}$ , cool, transfer to 500 ml volumetric flask, and dilute to mark. To 10 ml of the infusion (filtered, if not clear), add 25 ml of the indigo carmine soln and ca 750 ml of  $\text{H}_2\text{O}$ . Add the  $\text{KMnO}_4$  soln from a buret, a little at a time while stirring, until color becomes light green, then dropwise until color changes to bright yellow or to faint pink at rim. Designate number of ml of  $\text{KMnO}_4$  used as "a."

Mix 100 ml of the clear infusion of tea with 50 ml of the gelatin soln, 100 ml of the acid NaCl soln, and 10 g of powdered kaolin, and shake several minutes in stoppered flask. After allowing mixture to settle, decant thru filter. Mix 25 ml of filtrate with 25 ml of the indigo carmine soln and ca 750 ml of  $\text{H}_2\text{O}$  and titrate with  $\text{KMnO}_4$  as before. Number of ml of  $\text{KMnO}_4$  used subtracted from that obtained above, "a," gives quantity of  $\text{KMnO}_4$  required to oxidize the tannin. 1 ml of 0.1 N oxalic acid = ca 0.0042 g of tannin (gallotannic acid).

## FACING

44

GENERAL<sup>11</sup>—TENTATIVE

(1) Examine ash obtained as directed under 30 for mineral pigments (cf. XXI, 1); (2) shake quantity of the tea with large volume of  $\text{H}_2\text{O}$  and remove leaves by means of sieve. Allow insoluble matter in the  $\text{H}_2\text{O}$  portion to settle, filter, and examine residue on filter paper for insoluble pigments as directed under XXI, 1. Catechu and other soluble substances, if used, will be found in filtrate.

45

## PARAFFIN AND WAXY SUBSTANCES—TENTATIVE

Spread a quantity of the tea between two sheets of unglazed white paper and place thereon a hot iron. Any greasy substance will stain the paper.<sup>12</sup>

46

PIGMENTS USED FOR COLORING OR FACING<sup>12</sup>—TENTATIVE

Place 60 g of the tea in 60-mesh, 5-6" sieve provided with top. Sift small quantity (ca. 0.1 g) of the dust upon piece of semi-glazed, white paper ca 8 by 10". (To obtain

requisite quantity of dust, it is sometimes necessary to rub leaf gently against bottom of sieve.) Place paper on plain, firm surface, preferably glass or marble, and crush dust by pressing firmly upon it flat steel spatula ca 5" long. Repeat crushing process until tea dust is ground almost to powder, when particles of coloring matter, if present, become visible as streaks on the paper. Brush off loose dust and examine paper by means of simple lens magnifying 7.5 diameters. Bright light is essential to distinguish these particles and streaks. In many cases the character of the pigment is indicated by the behavior of these streaks when treated with reagents and examined under a microscope. The crushed particles of leaf of either black or green tea appear in such quantity that there is no chance of mistaking them for coloring or facing material. Repeat this test, using black, semi-glazed paper for facings such as talc, gypsum, BaSO<sub>4</sub>, or clay.

## SELECTED REFERENCES

- <sup>1</sup> J. Am. Chem. Soc., 41, 1298 (1919); J. Assoc. Official Agr. Chem., 5, 267 (1921); 13, 265 (1930); 14, 533 (1931); 16, 567 (1933); 17, 380 (1934).
- <sup>2</sup> Z. Nahr. Genussm., 28, 9 (1914); J. Assoc. Official Agr. Chem., 4, 526 (1921).
- <sup>3</sup> J. Assoc. Official Agr. Chem., 3, 498 (1920).
- <sup>4</sup> Forsch. Lebensm., 2, 223 (1895).
- <sup>5</sup> U. S. Dept. Agr. Div. Chem. Bull. 13 (VII); Villiers, Collin, and Fayolle, *Traité des Falsifications et Altérations des Substances Alimentaires*, 2nd ed. (1909-11).
- <sup>6</sup> U. S. Dept. Agr. Misc. Circ. 9, Reg. 18.
- <sup>7</sup> J. Assoc. Official Agr. Chem., 7, 154 (1923).
- <sup>8</sup> J. Am. Chem. Soc., 41, 1298 (1919); J. Assoc. Official Agr. Chem., 5, 288 (1921); 6, 107 (1922).
- <sup>9</sup> J. Assoc. Official Agr. Chem., 5, 291 (1921); 6, 107 (1922).
- <sup>10</sup> U. S. Dept. Agr. Div. Chem. Bull. 13 (VII), p. 890.
- <sup>11</sup> U. S. Dept. Agr. Bur. Chem. Bull., 90, p. 38.
- <sup>12</sup> U. S. Treas. Dept. T. D. 35244, March 23, 1915; U. S. Dept. Agr. Misc. Circ., 9, Reg. 23.
- <sup>13</sup> U. S. Treas. Dept. T. D. 35244, March 23, 1915; Proc. Eighth Intern. Cong. Appl. Chem., 18, 301 (1912); U. S. Dept. Agr. Misc. Circ. 9, Reg. 24.

## XIX. CACAO BEAN AND ITS PRODUCTS

1

### PREPARATION OF SAMPLE—OFFICIAL

Mix powdered products thoroly and preserve in tightly stoppered bottles. Chill sweet or bitter chocolate until it becomes hard and reduce to fine granular condition by grating or shaving. Mix thoroly and preserve in tightly stoppered bottle in cool place.

2

### MOISTURE—TENTATIVE

Dry 2 g of prepared sample, 1, to constant weight in Pt dish in air oven at 100°. (Al dish may be used when ash is not determined on same sample.) Report loss in weight as moisture.

3

### ASH—OFFICIAL

Proceed as directed under XXXIV, 9 or 10, using sufficient sample to contain ca 1 g of moisture-, sugar-, and fat-free material.

4

### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using ash obtained under 3.

5

### ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using filtrate from 4.

6

### ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 15, using insoluble ash obtained under 4.

7

### ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under XXXIII, 5, using total ash obtained under 3, or water-insoluble residue obtained under 4.

8

### TOTAL NITROGEN—OFFICIAL.—See II, 21, 22, or 23.

9

### MILK PROTEINS—TENTATIVE

Weigh exactly 10 g of finely grated chocolate into suitable 8 oz. centrifuge bottle. Add two 100 ml portions of ether, shake, centrifuge, and decant supernatant liquor after each addition. Dry residue in oven at ca 100° and powder residue in bottle with flattened glass rod. Add 200 ml of 3%  $\text{Na}_2\text{C}_2\text{O}_4$  and let stand 4 hours, shaking frequently. Centrifuge and filter thru small folded filter. Discard first 5–10 ml of filtrate and determine N in 50 ml of this filtrate. Pipet 100 ml of filtrate into 200 ml volumetric flask and dilute almost to mark with  $\text{H}_2\text{O}$ . Precipitate proteins by addition of 2 ml of glacial acetic acid. Make to volume, shake, filter, and determine N in 100 ml of filtrate. Difference between the two N figures obtained is the N of the casein contained in 2.5 g of the sample. This figure  $\times 4 \times 6.38$  = total casein contained in the 10 g taken for analysis. Casein  $\times 1.25$  = total milk protein.

10

### SUCROSE

Transfer 26 g of prepared sample, 1, to 8 oz nursing bottle, add ca 100 ml of petroleum benzin, shake 5 min., and centrifuge. Decant clear solvent carefully and repeat treatment with petroleum benzin. Place bottle containing defatted residue in warm place until petroleum benzin is expelled. Add 100 ml of  $\text{H}_2\text{O}$  and shake until

most of chocolate is detached from sides and bottom of bottle. Loosen stopper and carefully immerse bottle 15 min. in water bath kept at 85–90°, shaking occasionally to remove all chocolate from sides of bottle. Remove from water bath, cool, and add basic Pb acetate soln (sp. gr. 1.25) to complete precipitation (5 ml is usually sufficient). Add H<sub>2</sub>O to make total volume of 110 ml of added liquid. Mix thoroly, centrifuge, and decant the supernatant liquid thru small filter. Precipitate excess of Pb with powdered dry K oxalate and filter. Dilute sufficient filtrate with an equal volume of H<sub>2</sub>O, mix, and polarize in 200 mm tube at 20°, "P." Obtain invert reading, "I," at 20° as directed under XXXIV, 24(b). Multiply both readings by 2 to correct for dilution. From data obtained calculate percentage of sucrose (S) from following formulas:

$$S = \frac{(P-I)(110+X)}{143.0-t/2}, \text{ in which}$$

$$X = \frac{0.2244(P-21d)}{1-0.00204(P-21d)}, \text{ in which}$$

$$d = \frac{P-I}{143.0-t/2}.$$

## 11

LACTOSE IN MILK CHOCOLATE<sup>3</sup>

Determine reducing sugars before inversion as directed under XXXIV, 38, in aliquot (usually 20 ml) of the Pb-free filtrate obtained in 10. Determine reduced Cu as Cu<sub>2</sub>O by the volumetric thiosulfate method, XXXIV, 41. Correct for Cu<sub>2</sub>O due to sucrose as follows: Obtain approximate percentage of lactose from following formula, using data obtained in 10.

$$\text{Approximate lactose} = \frac{P(1.1+X/100)-S}{0.79}.$$

From calculated polarimetric sucrose/lactose ratio and total Cu<sub>2</sub>O obtained as above, determine amount of Cu<sub>2</sub>O to be subtracted from total Cu<sub>2</sub>O found, using plot (Fig. 23). Convert corrected Cu<sub>2</sub>O to lactose (L), using Table 9, XLIII. Percentage of lactose is then obtained from following relationship:

$$\text{Percentage lactose} = \frac{L(110+X)}{0.26C},$$

in which  $X$  = value obtained in polarimetric sucrose determination and  $C$  = volume of soln (ml) used in above lactose determination.

## FAT

## 12

Method I<sup>4</sup>—Official

Prepare in Knorr extraction tube a tightly packed mat of asbestos purified as for determination of crude fiber, XXVII, 25(c), and carefully freed from coarse pieces. Wash filter with alcohol, ether, and a little petroleum benzin. (All petroleum benzin used in this determination must be redistilled below 60°.) Weigh 2–3 g of prepared sample, 1, into tube and insert tube into rubber stopper in filtering bell-jar connected to suction thru two-way stopcock, taking care that no rubber particles adhere to tip of stem. Place weighed 150 ml Erlenmeyer flask at such height that the tube stem passes thru neck into flask. (Stem of tube should be lengthened if necessary.) Fill tube to ca  $\frac{2}{3}$  of capacity with the redistilled petroleum benzin, and

by means of rod having a flattened end stir sample thoroly, taking care to crush all lumps. Let stand 1 min. and drain by suction. Regulate suction so that collected solvent in flask will not boil violently. Add solvent from wash-bottle, at same time turning tube between thumb and finger so that sides of tube are washed down by each addition. Repeat extractions, with stirring, until fat is removed (10 extractions usually). Remove tube with stopper from bell-jar, wash traces of fat from end of stem with petroleum benzin, evaporate solvent, and dry to constant weight at 100°.

The fat-free sample may be used for crude fiber determination.

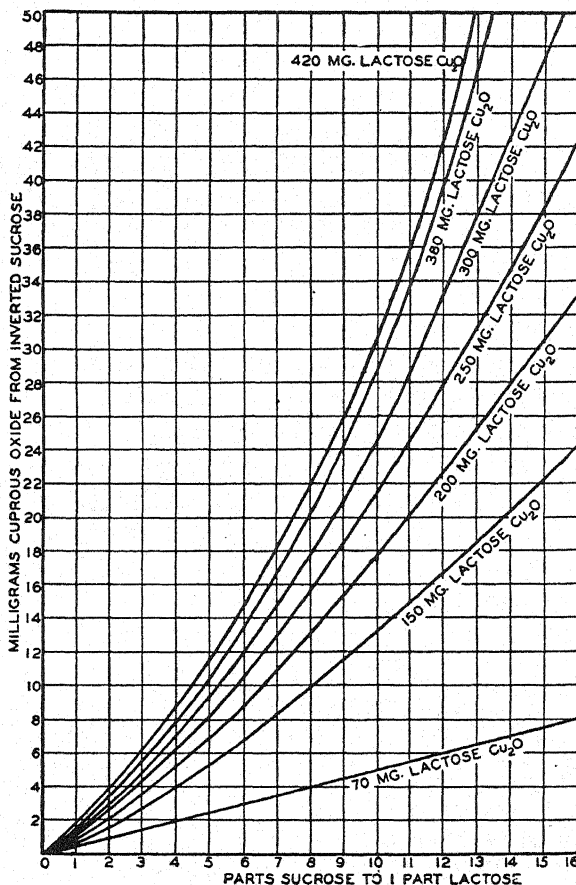


FIG. 23.—GRAPH USED IN CORRECTING CUPROUS OXIDE FOR EFFECT OF SUCROSE

13

#### Method II<sup>b</sup>—Tentative

Weigh accurately ca 2 g of prepared sample, 1, and without previous drying stratify charge in extraction tube with ca 0.5 g of asbestos, XXVII, 25(c), further washed with alcohol, ether, and petroleum benzin. Extract with petroleum benzin, redistilled below 60°, in continuous extractor 4 hours. Grind material to break up any lumps that may have formed and re-extract at least 4 hours. (It is advisable to allow solvent to run thru material once completely before applying heat for

continuous extraction.) Collect petroleum benzin extract in weighed flask, evaporate solvent, and dry residue to constant weight at 100°. Extracted residue in extraction tube may be used for determination of crude fiber.

## 14

## MILK FAT IN MILK CHOCOLATE—TENTATIVE

Estimate quantity of milk fat in milk chocolate from following formula, based on Reichert-Meissl number of 0.5 for cacao butter:

$$C = \frac{24A + 0.5B}{5}, \text{ in which}$$

$A$  = g of butter fat in 5 g of mixed fat;

$B$  = 5 -  $A$  = g of cacao fat in 5 g of mixed fat; and

$C$  = Reichert-Meissl number of extracted fat.

From which the

$$\text{Weight of butter fat in 5 g of mixed fat} = \frac{C - 0.5}{4.7}, \text{ and the}$$

$$\text{Percentage of butter fat} = \text{percentage of total fat} \times \frac{C - 0.5}{23.5}.$$

## 15

## SEPARATION AND PREPARATION OF FAT—TENTATIVE

Separate fat from 10–40 g of sample (depending upon fat content) by shaking material with 2 or 3 100 ml portions of ethyl ether. Centrifuge, and decant each portion. Combine portions in beaker and drive off most of ether on steam bath. Filter ether extracts thru dry, folded filter and dry at 100°.

16 DETECTION OF COCONUT AND PALM KERNEL OILS IN CACAO BUTTER AND FAT EXTRACTED FROM MILK CHOCOLATE<sup>6</sup>—TENTATIVE

(a) *Examination of cacao butter.*—Saponify 5 g of sample with 15 ml of alcoholic KOH soln (25 g to 200 ml of alcohol) and evaporate alcohol on steam bath. Run blank on pure cacao butter at same time. Add 5 ml of H<sub>2</sub>O and again evaporate to remove last trace of alcohol. Dissolve soap in 100 ml of H<sub>2</sub>O, cool to room temp., and add, while stirring, 100 ml of saturated salt soln. Allow to stand 15 min., stirring several times during this period, and then separate the soap by filtration, using Büchner funnel. To 100 ml of filtrate add, while stirring, 100 ml of the saturated salt soln and allow to stand 15 min. (Only a slight precipitate should appear.) Filter, add to filtrate<sup>1</sup> a drop of phenolphthalein indicator, neutralize with HCl (1+3), and add 0.5 ml of this reagent in excess. If sample consists of pure cacao butter, soln when acidified will remain clear; if coconut or palm kernel oil is present, soln will become turbid or milky.

(b) *Examination of fat extracted from milk chocolate.*<sup>7</sup>—Milk fat, if present in cacao butter subjected to this test, produces a turbidity less in intensity than that produced by same percentage of coconut or palm kernel oil. For example, cacao butter containing 10, 15, or 20% of milk fat produces, respectively, no opalescence, faint opalescence, or an opalescence. For this reason, when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply percentage of lactose in cacao product by 0.8, or percentage of casein by 1.1, to obtain percentage of milk fat in the product, and from this result calculate percentage of milk fat in total fat. If this percentage corresponds to 15% or less, a blank of cacao butter containing 15% milk fat may be used; otherwise make up mixture of cacao butter and milk fat in proportions indicated by the calculations.

Test fat extracted from sample under examination as directed under (a), but use the prepared mixture of cacao butter and milk fat instead of the pure cacao butter for the blank. If fat being tested contains coconut oil or palm kernel oil, the last filtrate, when acidified, will be more turbid or milky than the blank.

#### CRITICAL TEMPERATURE OF DISSOLUTION OF FAT IN ACETIC ACID<sup>a</sup>—TENTATIVE

17

##### APPARATUS

Insert thermometer reading to  $0.1^{\circ}$  into cork that fits a  $6 \times \frac{3}{4}$ " test tube and extend it far enough into tube so that bulb will be covered by 10 ml of liquid. Place test tube in larger tube ( $4 \times 1\frac{1}{4}$ " ) containing glycerol and hold firmly in place with cork having groove cut in side to equalize pressure when heat is applied.

18

##### DETERMINATION

To remove traces of moisture, filter portion of sample to be examined thru dry paper in oven in which a temp. of ca  $110^{\circ}$  is maintained. Allow filtered sample to cool until barely warm and weigh 5 g of the sample and 5 g of 99.5% acetic acid into the test tube. Insert cork holding thermometer and place test tube in glycerol bath. Heat, and shake apparatus frequently until a clear soln of the fat and acetic acid is obtained. Allow soln to cool, with constant shaking, without removing from glycerol bath. Note temp. at which first sign of turbidity appears. Make similar test with the same acetic acid on sample of pure cacao butter.

As free fatty acids lower turbidity temp., a correction must be made for the acid value of the sample. If the strength of the acetic acid reagent is such that the turbidity temp. of the pure cacao butter is ca  $90^{\circ}$ , one unit of acid value will cause a reduction of  $1.4^{\circ}$  in the critical temp. of dissolution. If the turbidity temp. is ca  $100^{\circ}$ , one unit of acid value will cause a reduction of  $1.2^{\circ}$ . For intermediate temp. reduction is proportional.

Determine acid value (mg of KOH required to neutralize free fatty acids in 1 g of sample) of both sample and pure cacao butter as directed under XXXI, 32, using 5 g of fat. Multiply the acid value by correction factor and add result to observed turbidity temp. The figure obtained is the true critical temp. of dissolution. If this temp. is lower than that of pure cacao butter by more than  $3^{\circ}$  in the case of fat from chocolate liquors or sweet chocolates, and by more than  $6^{\circ}$  in the case of fat from milk chocolates, adulteration with coconut, palm kernel, corn, peanut, cottonseed oils, etc., or their stearins, is indicated.

19

#### ACETONE-CARBON TETRACHLORIDE TEST OF FAT<sup>a</sup>—TENTATIVE

Dissolve 5 ml of the warm fat, which has been previously filtered thru dry filter paper in oven at ca  $110^{\circ}$  to remove traces of moisture, in 5 ml of acetone- $\text{CCl}_4$  reagent (equal quantities of each) in test tube. Allow soln to stand in ice  $\text{H}_2\text{O}$  20–30 min. Run blank on sample of pure cacao butter at same time. If hydrogenated oil, tallow, oleostearin, or paraffin is present, a white flocculent precipitate will soon appear. If the  $\text{H}_2\text{O}$  is cold enough, cacao butter may solidify. If precipitate is formed, remove sample from ice  $\text{H}_2\text{O}$  and allow to remain at room temp. for a time. Solidified cacao butter will soon melt and go into soln, but if precipitate is due to any of the above-mentioned possible adulterants a much longer time will be required.

20

#### MELTING POINT—OFFICIAL

Proceed as directed under XXXI, 14. Keep fat at least 24 hours in cool place before making determination.



- 21 INDEX OF REFRACTION—OFFICIAL.—See XXXI, 9.
- 22 IODINE ABSORPTION NUMBER—OFFICIAL.—See XXXI, 19 OR 21.
- 23 SAPONIFICATION NUMBER—OFFICIAL.—See XXXI, 25.
- 24 REICHERT-MEISSL AND POLENSKE VALUES<sup>a</sup>—OFFICIAL.—See XXXI, 29
- SILVER NUMBER FOR DETECTION OF COCONUT AND PALM KERNEL OILS<sup>b</sup>—TENTATIVE

25 REAGENTS

- (a) *Potassium hydroxide soln.*—750 g of KOH per liter.
- (b) *Magnesium sulfate soln.*—150 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  per liter.
- (c) *Sodium nitrate.*—Crystals as Cl-free as practicable (0.002% or less).
- (d) *Ferric indicator.*—Saturated. Use ferric potassium sulfate or ferric ammonium sulfate.

26 DETERMINATION

Weigh 10 g of fat into 250 ml beaker and add 40 ml of alcohol and 5 ml of the KOH soln. Saponify mixture and evaporate to dryness on steam bath. Take up soap in  $\text{H}_2\text{O}$  (150 ml), warming if necessary. Cool, and make up to 250 ml.

Pipet 200 ml of the soln into 500 ml Erlenmeyer flask. Close flask with stopper carrying thermometer and having small groove lengthwise in side. Place flask in water bath maintained at ca  $80^\circ$ . When sample reaches ca  $80^\circ$ , loosen stopper and introduce 50 ml of the  $\text{MgSO}_4$  soln from pipet. Shake flask with rotary motion. Replace stopper and thermometer and allow flask to remain in bath 8–10 min. longer at  $70$ – $80^\circ$ , shaking flask occasionally. Remove flask and cool under tap, with shaking, to  $20$ – $25^\circ$ . Remove stopper and thermometer, stopper tightly, and shake vigorously 4 min. Allow flask to stand in bath at  $20$ – $25^\circ$  until a water layer separates at bottom. Filter thru Büchner funnel, removing all liquid possible by pressing with a horn spoon. Run blank on cacao butter in same manner.

Neutralize 200 ml of filtrate until colorless to phenolphthalein with ca 0.5 N  $\text{H}_2\text{SO}_4$  soln in 250 ml volumetric flask. Add 20 g of the  $\text{NaNO}_3$  crystals and when dissolved add 22.5 ml of 0.2 N  $\text{AgNO}_3$  soln. Make to mark and shake 3 min. Allow flask to stand short time and filter thru folded filter. To 200 ml of filtrate add 6 ml of the ferric indicator and 4 ml of 40%  $\text{HNO}_3$ . Titrate with 0.1 N  $\text{NH}_4\text{SCN}$  to first color change (reddish brown).

Calculate as follows:

Silver number (mg of silver used per g of fat) =  $(a - b) \times 2.107$ , in which

$a$  =  $1.6 \times \text{ml of } 0.2 \text{ N silver nitrate soln added}$ ; and

$b$  = ml of 0.1 N  $\text{NH}_4\text{SCN}$  soln used in back titration.

$$\text{Factor } 2.107 = \frac{10.788 \text{ (mg Ag per ml } 0.1 \text{ N soln)}}{5.12 \text{ (g of fat in aliquot titrated)}}$$

The silver number of palm kernel and coconut oils and stearins varies from ca 26 for the stearins to 60 for whole coconut oil. Dairy butter gives a value of ca 11.6, and cacao butter, 0.6.

27 CRUDE FIBER<sup>11</sup>—OFFICIAL

(For cacao products except milk chocolate.)

Treat 7 g of liquor or 50 g of sweet chocolate in nursing bottle with two 100 ml portions of ether, centrifuging and decanting supernatant liquor after each addition. Dry residue in oven at ca  $100^\circ$  and then powder in bottle with flattened glass rod.

(In some cases it may be necessary to grind material in mortar and extract third time with ether.) Wash mixture in nursing bottle with three 100 ml portions of  $H_2O$  at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge after each washing 10–15 min., and decant aqueous layer. Wash residue in same fashion with two 100 ml portions of alcohol and one 100 ml portion of ethyl ether. Transfer residue to Pt dish, dry to constant weight, and grind in mortar. Weigh 2 g of dried material and determine percentage of crude fiber ( $D$ ) as directed under XXVII, 27, using linen for both acid and alkaline filtrations. Calculate percentage of crude fiber on moisture-, fat- and sugar-free basis ( $E$ ) by the formula  $E = 0.7D$ .

28

## CRUDE FIBER IN MILK CHOCOLATE—OFFICIAL

Treat 50 g of milk chocolate with three 100 ml portions of ether in nursing bottle, centrifuging and decanting supernatant liquor after each addition. Dry residue in the bottle and powder with flattened glass rod. Shake with 100 ml of 1%  $Na_2C_2O_4$  soln, and let stand 30 min. Centrifuge and decant supernatant liquor. Wash in nursing bottle with three 100 ml portions of  $H_2O$  at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge after each washing 10–15 min. and decant aqueous layer. Wash residue in same fashion with two 100 ml portions of alcohol and one 100 ml portion of ethyl ether. Transfer residue to Pt dish, dry to constant weight at  $100^\circ$ , and grind in mortar. Weigh 2 g of the dried material and determine percentage of crude fiber as directed in XXVII, 27, using linen for both acid and alkaline filtrations. Percentage of crude fiber found  $\times 0.7$  = percentage of crude fiber on true fat-, sugar-, moisture- and milk-free basis.

## STARCH

29

*I. Direct Acid Hydrolysis Method—Tentative*

Weigh 4 g of sample if unsweetened, or 10 g if sweetened, into small porcelain mortar; add 25 ml of ether and grind. After coarser material has settled, decant ether, together with fine suspended matter, on 11 cm paper of sufficiently fine texture to retain crude starch. Repeat this treatment until no more coarse material remains. After ether has evaporated from filter, transfer fat-free residue to mortar by means of jet of cold  $H_2O$  and rub to even paste, filtering on paper previously used. Repeat this process until all sugar is removed. In case of sweetened products filtrate should measure at least 500 ml. Determine crude starch in extracted residue as directed under XXVII, 30.

30

*II. Diastase Method—Tentative*

Remove fat and sugar from 4 g of sample if unsweetened, or 10 g if sweetened, as directed under 29. Carefully wash wet residue into beaker with 100 ml of  $H_2O$ , heat to boiling over asbestos with constant stirring, and continue the boiling and stirring 30 min. Replace  $H_2O$  lost by evaporation and immerse beaker in water bath kept at  $55$ – $60^\circ$ . When liquid has cooled to temp. of bath, add 20 ml of freshly prepared malt extract, XXVII, 31, and digest mixture 2 hours with occasional stirring. Boil a second time for 30 min., dilute, cool, and digest as before with another 20 ml portion of the malt extract. Heat again to boiling, cool, and transfer to 250 ml flask. Add 3 ml of alumina cream, dilute to mark, and filter thru dry paper. The residue on the paper should show no signs of starch when examined microscopically. Continue from this point as directed under XXVII, 32, beginning "Place 200 ml of filtrate in flask, add 20 ml of  $HCl$  (sp. gr. 1.125)."

31

COLORING MATTERS—TENTATIVE.—See XXI, 2(e)

32

SHELL IN CACAO NIBS—TENTATIVE

## TRIER FOR SAMPLING

Use a double-tube, separate-compartment grain trier to collect samples of nibs from bins, trucks, and sacks. The tubes of the trier are of No. 16 gage (B & S) (0.0508") seamless metal. The outside diameter of outer tube is  $1\frac{3}{8}$ ", and outer and inner tubes fit each other closely. The width of openings in outer tube is  $15/16$ ", and in the inner tube 1". The length of such openings in both tubes is  $3\frac{1}{2}$ ", except that the length of the opening of compartment nearest point of trier may be  $3-3\frac{1}{2}$ ". Each compartment coincides with and is of same length as its opening in the inner tube. The openings of inner and outer tubes match when trier is open for sampling. The distance between adjacent compartments is  $1\frac{1}{2}-2$ ", and distance between point of trier and compartment end nearest point is not more than  $1\frac{1}{8}$ ".

33

## COLLECTION OF SAMPLE

(a) *From bins or trucks.*—Collect a sample of ca 10 lbs. by probing with the trier, 32. Probe nibs to floor of bin or truck, spacing individual probings approximately equidistant from each other thruout top area of nibs. If contents of bin are inaccessible, or are of greater depth than length of trier from its point to 2" above compartment end nearest handle, take sample from chute thru which bin is being filled or emptied as directed in (c).

(b) *From sacks.*—Collect sample of ca 10 lbs. by probing with the trier, 32. The length of trier from its point to 2" above compartment end nearest handle equals or exceeds depth to which sacks are filled. Probe with trier thru entire depth of nibs in sack. Probe a number of sacks equal to at least the square root of total number of sacks in lot. If lot is of less than 12 sacks probe at least  $\frac{2}{3}$  of them; if of more than 12 probe at least 8.

(c) *From chutes.*—Collect a sample of ca 10 lbs. by catching momentarily and at regular intervals in suitable receptacle a cross section of the stream of nibs from chute. Continue sampling thruout time the lot of nibs being sampled is passing thru the chute.

34

## REDUCTION OF SAMPLE

Using a sample divider of type described in U. S. Department of Agriculture Bull. No. 287, September 14, 1915, and No. 857, June 25, 1920, reduce size of sample collected, 33, to ca  $\frac{1}{2}$  lb. Weigh reduced sample to nearest 0.05 g.

35

## DIVISION OF SAMPLE

(a) *Hand division.*—Screen reduced sample, 34, in successive portions of 75–100 g, on a circular sieve, the bottom of which is No. 10 woven wire cloth that complies with specifications for such cloth set forth on page 3 of the publication, "Standard Specifications for Sieves," October 25, 1938, U. S. Department of Commerce, National Bureau of Standards. The diameter of sieve is ca 8". Collect material remaining on sieve and designate it as L. Screen material that passed thru sieve on another circular sieve, the bottom of which is No. 20 wire cloth that complies with specifications for such cloth set forth on the same page of such publication. The diameter of sieve is either 6 or 8". Collect portion remaining on sieve and designate it as S. Collect material passing thru sieve and designate it as F. Treat portions L, S, and F, respectively, as directed under 36(a), (b), and (c).

(b) *Machine division*.—Use a sample-size, grain-cleaning mill of type described on page 16 of U. S. Department of Agriculture Farmers' Bull. No. 1747, issued September 1935. Fit into lower slot of mill a single screen having circular openings 0.083–0.093" in diameter. The machine is provided with settling traps to catch all material blown out by fan. The inclined slide under screen is provided with a removable slat in such position that when it is removed the material passing thru screen is discharged from mill without going to fanning chamber. Remove this slat, start mill, and slowly pour reduced sample, 34, over upper part of screen. Designate as L' the material that does not pass thru screen and is not removed by fanning. Collect material that passes thru screen and screen it again on sieve having No. 20 woven wire cloth, 35(a). Collect portion remaining on sieve and designate it as S. Collect portion passing thru sieve and designate it as F. Reserve F for treatment as directed in 36(c).

Replace slat in mill and without removing L' or fannings, start mill and pour S slowly onto upper part of screen. Allow the material thus cleaned to combine with L', and designate combination as L' S'. Treat L' S' as directed in 36(a). Remove combined fannings from settling traps and screen on sieve having No. 10 wire cloth, described under 35(a). Collect portion remaining on sieve and designate it as LS. Collect portion passing thru sieve and designate it as SS. Treat LS as directed in 36(a), and SS as directed in 36(b).

## 36

## DETERMINATION

(a) Place L (from hand division), or L' S' (from machine division), on large sheet of sized paper. Scatter 2–3 g of L or 4–5 g of L' S' over an area of the paper ca 3–4" in diameter. Examine scattered portion and remove pieces of shell with spatula or tweezers. Examine entire portion of L or L' S' progressively in this manner. Separate shell from LS (from machine division) in same manner. Reserve separated shell for later combination with shell from other fractions.

(b) If S (from hand division) weighs more than 4.5 g and appears to contain large quantity of shell, weigh it accurately to nearest 10 mg; mix entire portion by pouring it gradually several times from one glazed paper to another, each time forming conical piles; flatten and quarter last pile formed, and combine alternate quarters; if necessary mix and again reduce by quartering until a weight of 3–4.5 g is obtained; and weigh fraction thus obtained accurately to nearest 10 mg. If quantity of shell in S appears to be small, use entire portion. Separate shell from S or fraction thereof and from SS (from machine division) as follows:

Place a blotting paper of ca 19×24" on a firm supporting plane inclined at an angle of 21–24° from horizontal. Pour all the material gradually and in successive portions from an elevation of 2–3" along upper end of blotter. Shake blotter slowly to cause nib material to roll down, and at intervals remove material collected at bottom. Toward end of procedure shake blotter more rapidly to detach most of nib material. After removing shell adhering to blotter repeat procedure on last portions of material collected at bottom of blotter. Using a reading glass, complete separation of shell and nibs with spatula or tweezers by examining portions until all material is examined. Except in separations from a fraction of S, reserve separated shell for combination with that obtained from other separations. In the case of separations from a fraction of S, weigh separated shell to nearest mg and calculate total weight of shell in S.

(c) Place F (obtained from either hand division or machine division) in 400 ml beaker about half full of a soln composed of 2½ volumes of CCl<sub>4</sub> and 1 volume of 96% alcohol. Stir mixture ca 1 min., but slowly at the last, and allow to stand 3–4

min. Skim off floating nibs with tea strainer made with approximately No. 40 wire cloth. Decant liquid and any suspended material from beaker without disturbing residue until 2-4 ml remains. Wipe inside of beaker above liquid with filter paper moistened in the solution described above so as to remove all nib material. Add ca 25 ml of petroleum benzin, b.p. 30-65°, swirl liquid in beaker a few times, allow residue of shell to settle, and carefully decant liquid. Allow remaining liquid to evaporate and dry the shell on steam bath. Reserve shell for combination with other fractions of shell.

(d) Combine all shell obtained from L, S, and F (from hand division) or L' S', LS, SS, and F (from machine division) and weigh combined shell from reduced sample. If a fraction of S was used, combine L and F, weigh, and add calculated weight of shell in S to obtain weight of shell from reduced sample. Report result in terms of percentage by weight of shell in nibs.

#### SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., **16**, 563 (1933); **17**, 64 (1934).
- <sup>2</sup> Ibid., **16**, 565 (1933); **17**, 65 (1934).
- <sup>3</sup> Ibid., **16**, 566 (1933); **17**, 65, 379 (1934).
- <sup>4</sup> Ibid., **9**, 46 (1926).
- <sup>5</sup> Ibid., **10**, 42 (1927).
- <sup>6</sup> Ibid., **11**, 45 (1928).
- <sup>7</sup> Ibid., **13**, 45, 78, 486 (1930).
- <sup>8</sup> Ibid., **5**, 263 (1921); **7**, 150 (1923).
- <sup>9</sup> Ibid., **13**, 43 (1930).
- <sup>10</sup> Ibid., **15**, 549 (1932); **17**, 64, 375 (1934).
- <sup>11</sup> Ibid., **14**, 530 (1931); **16**, 66 (1933).

## XX. CEREAL FOODS

### WHEAT FLOUR<sup>1</sup>

1

#### DIRECTIONS FOR SAMPLING—OFFICIAL

Sample a number of sacks equivalent to square root of number in lot, but not less than 10, *i.e.*, 10 from 100 or less, 15 from 225, 20 from 400 sacks, etc.

Select sacks to be sampled according to their exposure in ratio of 4 from most exposed, 3 from next less exposed, 2 from next, and 1 from the least exposed portion of the lot.

From each sack to be sampled, draw a core from one corner of top diagonally to center of sack by means of cylindrical, pointed, polished metal trier,  $\frac{1}{2}$ " in diameter, with slit at least  $\frac{1}{4}$  the circumference. Draw a second core from other top corner to  $\frac{1}{2}$  distance to center of sack.

Deliver the 2 cores at once to a clean, dry, air-tight container which has stood open for a few minutes near the lot of flour to be sampled and seal immediately. Use separate container for each sack sampled. One of following containers may be used: (1) Pint fruit jar provided with rubber gasket; (2) rubber pouch which can be tied or sealed to exclude moisture or air; (3) tin can or box with moisture and air-tight friction top.

Before opening sample for analysis, alternately invert and roll each container 25 times, or more if necessary, to secure homogeneous mixture. Avoid extreme temp. and humidities when opening containers for analysis. Keep sample tightly sealed at all other times.

#### TOTAL SOLIDS (MOISTURE, INDIRECT METHOD)

##### *Vacuum Oven Method<sup>2</sup>—Official*

2

#### APPARATUS

(a) *Metal dish*.—Diameter ca 55 mm, height ca 15 mm, provided with inverted slip-in cover fitting tightly on inside.

(b) *Air-tight desiccator*.—Reignited quick lime is a satisfactory drying agent.

(c) *Vacuum oven*.—Connect with pump capable of maintaining partial vacuum in oven with pressure equivalent to 25 mm or less of Hg and provided with thermometer passing into oven in such a way that bulb is near samples. Connect H<sub>2</sub>SO<sub>4</sub> gas-drying bottle with oven for admitting dry air when releasing vacuum.

3

#### DETERMINATION

Weigh accurately ca 2 g of the well-mixed sample in covered dish that previously has been dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen the cover (do not remove) and heat at 98–100° to constant weight (ca 5 hours) in partial vacuum having pressure equivalent to 25 mm or less of Hg. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover on dish, transfer to desiccator, and weigh soon after room temp. is attained. Report flour residue as total solids and loss in weight as moisture (indirect method).

4

##### *Air-Oven Method<sup>2</sup>—Official*

(Results closely approximate those obtained by vacuum method.)

In a cooled and weighed dish (provided with cover) that has been previously

heated to  $130^{\circ} \pm 3^{\circ}$ , weigh accurately ca 2 g of the well-mixed sample. Uncover sample and dry dish, cover, and contents an hour in an oven provided with opening for ventilation and maintained at  $130^{\circ} (\pm 3^{\circ})$ . (1 hour drying period begins when oven temperature is actually  $130^{\circ}$ .) Cover dish while still in oven, transfer to desiccator, and weigh soon after room temp. is attained. Report flour residue as total solids and loss in weight as moisture (indirect method).

ASH<sup>4</sup>

5

*Method I—Official*

Weigh 3–5 g of the well-mixed sample into a shallow, relatively broad ashing dish that has been ignited, cooled in desiccator, and weighed soon after attaining room temp. Incinerate in furnace at ca  $550^{\circ}$  (dull red) until light gray ash results, or to constant weight. Cool in desiccator and weigh soon after room temp. is attained. Reignited quick lime is a satisfactory drying agent for desiccator.

*Method II—Magnesium Acetate Method<sup>5</sup>—Official*

6

## REAGENT

*Magnesium acetate soln.*—Dissolve 4.054 g of  $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$  in 50 ml of  $\text{H}_2\text{O}$  and make up to 1 liter with alcohol.

7

## DETERMINATION

From a buret add 5 ml of the reagent to 3–5 g of flour, bread, etc., or 10 ml to 1 g sample of bran, wheat germ, etc. Allow mixture to stand 1–2 min., evaporate excess alcohol, and place sample in muffle furnace maintained at  $700^{\circ}$ , closing door after flaming has ceased. When incineration is complete, place ashing dish in desiccator until cool, then weigh. Run blank determination on soln and deduct blank from weight of crude ash. Evaporate blank cautiously.

ORIGINAL ASH OF FLOUR IN PHOSPHATED AND SELF-RISING FLOUR<sup>6</sup>

8

*Gustafson Method—Tentative*

To 20–25 g of the flour in metal centrifuge tube (cup 2" in diameter, 6" deep), add sufficient  $\text{CCl}_4$  to fill tube to within 1" of top (ca 250 ml). Centrifuge 5–7 min. at 1,600 r.p.m., and allow centrifuge to come to rest slowly. Carefully skim off flour, which is now in compact layer on surface of the  $\text{CCl}_4$ , with large tablespoon, recovering as much of the flour as is possible in one spoonful. (With care, ca 90% of original flour may be recovered.) Allow wet flour to dry overnight and proceed as directed under 5. (The  $\text{CCl}_4$  may be filtered, distilled, and used again.)

9

TOTAL CARBON DIOXIDE IN SELF-RISING FLOUR<sup>6</sup>—OFFICIAL, FIRST ACTION

Use 17 g of flour, 15–20 glass beads (4–6 mm diam), and 45 ml of  $\text{H}_2\text{SO}_4$  (1+5), and proceed as directed under XVII, 4–6, as far as the calculation, except to agitate the flask vigorously 3 min. and allow it to stand 10 min. to secure equilibrium. Calculate as follows: Subtract volume of acid used from total buret reading and correct for temp. and pressure. Divide corrected reading by 100 to obtain percentage of  $\text{CO}_2$  (by weight). Correct apparent per cent of  $\text{CO}_2$  to compensate for varying atmospheric conditions by immediately assaying a synthetic sample of known composition and like ingredients by the same method in the same apparatus. Divide weight of  $\text{CO}_2$  recovered from synthetic sample by weight of  $\text{CO}_2$  contained in  $\text{NaHCO}_3$  used and record quotient. Apparent per cent of total  $\text{CO}_2$  in official sample  $\div$  this quotient = corrected per cent total  $\text{CO}_2$  in official sample.

10

## CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Proceed as directed under XXVII, 22. With fine flour the addition of an equal weight of clean, dry sand may be necessary.

11

FAT (ACID HYDROLYSIS METHOD)<sup>7</sup>—OFFICIAL

Place 2 g of the flour in 50 ml beaker, add 2 ml of alcohol, and stir so as to moisten all particles. (Moistening of sample with alcohol prevents lumping on addition of the acid.) Add 10 ml of HCl (25+11), mix well, set beaker in water bath held at 70–80°, and stir at frequent intervals 30–40 min. Add 10 ml of alcohol and cool. Transfer mixture to Röhrig or Mojonnier fat extraction apparatus. Rinse beaker into extraction tube with 25 ml of ethyl ether in 3 portions and shake mixture well. Add 25 ml of redistilled petroleum benzin (b.p. below 60°) and mix well. Let stand until upper liquid is practically clear. Draw off as much as possible of ether-fat soln thru filter consisting of pledget of cotton packed just firmly enough in stem of funnel to allow free passage of ether into weighed 125 ml beaker-flask containing some porcelain chips or broken glass. Before weighing beaker-flask dry it and a similar flask as a counterpoise in drying oven at 100° and then allow it to stand in air to constant weight. Re-extract liquid remaining in tube twice, each time with only 15 ml of each ether. Shake well on addition of each ether. Draw off clear ether solns thru filter into same flask as before and wash tip of spigot, funnel, and end of funnel stem with a few ml of a mixture of the 2 ethers in equal volumes free from suspended H<sub>2</sub>O. Evaporate ethers slowly on steam bath, then dry fat in drying oven at 100° to constant weight (ca 90 min.). Remove flask and counterpoise from oven, allow to stand in air to constant weight (ca 30 min.), and weigh. (Owing to size of flask and nature of the material, there is less error by cooling in air than in desiccator.) Correct this weight by blank determination on reagents used. Report as % fat by acid hydrolysis.

12

## CRUDE FIBER—OFFICIAL.—See XXVII, 25–27

13

## FAT ACIDITY IN FLOUR—TENTATIVE.—See XXVII, 39.

## H-ION CONCENTRATION—OFFICIAL, FIRST ACTION

14

PREPARATION OF SULFONPHTHALEIN INDICATORS<sup>8</sup>

	A	pH
Bromocrescol green	14.3	3.8–5.4
Chlorophenol red	23.6	4.8–6.4
Bromothymol blue	16.0	6.0–7.6
Phenol red	28.2	6.8–8.4

A = ml of 0.01 N NaOH per 0.1 g of indicator to form monosodium salt. Dilute to 250 ml for 0.04% reagent.

15

## PREPARATION OF STOCK SOLUTIONS

Use recently boiled H<sub>2</sub>O.

(a) *Acid potassium phthalate soln.*—0.2 M. Dry the C. P. salt to constant weight at 110–115°. Contains 40.836 g of the salt in 1 liter of soln.

(b) *Monopotassium phosphate soln.*—0.2 M. Dry the C. P. salt to constant weight at 110–115°. Should contain 27.232 g of the salt in 1 liter of soln. The soln should be distinctly red with methyl red, and distinctly blue with bromophenol blue.

(c) *Boric acid—KCl soln.*—0.2 M. Dry the C. P. H<sub>3</sub>BO<sub>3</sub> to constant weight in desiccator over CaCl<sub>2</sub>. Dry the C. P. KCl in oven at 115–120° for 2 days. 1 liter should contain 12.405 g of H<sub>3</sub>BO<sub>3</sub> and 14.912 g of KCl.





(d) *Sodium hydroxide soln.*—0.2 *M*. Should be as free as possible from carbonate. Dissolve 100 g of NaOH in 100 ml of H<sub>2</sub>O, and allow to stand overnight till carbonate has settled. Pipet clear soln from sediment and dilute to a soln somewhat more concentrated than normal. Standardize this soln with an acid soln of known strength, or with a sample of KHC<sub>8</sub>H<sub>4</sub>O<sub>4</sub>. From this approximate standardization calculate the quantity required for a 0.2 *M* soln. Make required dilution with least possible exposure and pour soln into Pyrex glass bottle. Carefully standardize the soln. The simplest method of doing this is by means of the KHC<sub>8</sub>H<sub>4</sub>O<sub>4</sub>. 0.04084 g of KHC<sub>8</sub>H<sub>4</sub>O<sub>4</sub> = 1 ml of 0.2 *M* NaOH. It is preferable to use a factor with the soln rather than attempt adjustment to an exact 0.2 *M* soln. Use phenolphthalein as indicator.

## 16

## PREPARATION OF BUFFER SOLUTIONS

The maximum range of standard buffer solns usually needed in cereal work is pH 5.0–8.6. Prepare these from the following stock solns, and in each case dilute to 200 ml.

*Phthalate-NaOH Mixtures*

pH	0.2 <i>M</i> KH PHTHALATE (ML)	0.2 <i>M</i> NaOH (ML)
5.0	50	23.65
5.2	50	29.75
5.4	50	35.25
5.6	50	39.70
5.8	50	43.10
6.0	50	45.40
6.2	50	47.00

*KH<sub>2</sub>PO<sub>4</sub>-NaOH Mixtures*

pH	0.2 <i>M</i> KH <sub>2</sub> PO <sub>4</sub> (ML)	0.2 <i>M</i> NaOH (ML)
5.8	50	3.66
6.0	50	5.64
6.2	50	8.55
6.4	50	12.60
6.6	50	17.74
6.8	50	23.60
7.0	50	29.54
7.2	50	34.90
7.4	50	39.34
7.6	50	42.74
7.8	50	45.17
8.0	50	46.85

*H<sub>3</sub>BO<sub>3</sub>-KCl-NaOH Mixtures*

pH	0.2 <i>M</i> H <sub>3</sub> BO <sub>3</sub> , KCl (ML)	0.2 <i>M</i> NaOH (ML)
7.8	50	2.65
8.0	50	4.00
8.2	50	5.90
8.4	50	8.55
8.6	50	12.00

## 17

## PREPARATION OF COLORIMETRIC STANDARDS

Place 20 ml of the buffered soln in ampuls  $\frac{3}{4}$ " in diameter, or in test tubes of similar bore, and add 0.5 ml of indicator soln. Do not keep the ampuls or tubes longer than a few days unless sealed, because the buffer solns may spoil.

## 18

## METHOD FOR MEASURING pH

To 10 g of the sample add 100 ml of cool, recently boiled distilled  $H_2O$  and digest at  $25^\circ$  for 30 min., shaking occasionally during digestion period. Allow mixture to stand quietly for 15 min. and then decant supernatant liquid thru a folded, hardened, dry filter paper. Discard first 5 ml to come thru, but catch the next 60 ml, 20 ml in each of three tubes exactly like the tubes holding the colorimetric standards. Add 0.5 ml of the proper indicator to one tube and compare the resultant color with the prepared standards to get the pH.

A somewhat crude but helpful application of Walpole's principle to compensate for color and turbidity of sample can be made from a block of wood. Bore parallel and in pairs, 6 deep holes, each large enough to hold one color standard or sample tube. Place adjacent pairs as close together as possible without breaking thru intervening walls. Perpendicular to these holes and running thru each pair bore smaller holes, thru which the test tubes may be viewed. The center pair of test tubes holds soln to be tested plus indicator and also a water blank. At each side place the standards colored with the indicator, and back each by a sample of soln being tested. Place the light on the side of the comparator containing the two controls and water blank. If daylight is used, the light from the northern sky is best. If artificial light is used it must not be too brilliant and should be passed thru daylight type of glass. For average conditions a light intensity of 15–20 microamperes as registered thru a photronic cell is adequate.

REDUCING AND NON-REDUCING SUGARS IN FLOUR<sup>a</sup>—OFFICIAL, FIRST ACTION

## 19

## REAGENTS

(a) *Acid buffer soln.*—Make 3 ml of glacial acetic acid, 4.1 g of anhydrous Na acetate, and 4.5 ml of  $H_2SO_4$  to 1 liter with  $H_2O$ .

(b) *Sodium tungstate.*—12%. Make 12.0 g of  $Na_2WO_4 \cdot 2H_2O$  to 100 ml with  $H_2O$ .

(c) *Ferricyanide soln.*—Alkaline 0.1 N. 33.0 g of pure dry  $K_3Fe(CN)_6$  and 44.0 g of anhydrous  $Na_2CO_3$  per liter.

(d) *Acetic acid-salt mixture.*—Make up 200 ml of glacial acetic acid, 70 g of KCl, and 40 g of  $ZnSO_4 \cdot 7H_2O$  to 1 liter with  $H_2O$ .

(e) *Soluble starch-potassium iodide soln.*—Add 2 g of soluble starch to small quantity of cold  $H_2O$  and pour slowly into boiling  $H_2O$  with constant stirring. Cool thoroly (or resulting mixture will be dark colored), add 50 g of KI and make up to 100 ml with  $H_2O$ . Add 1 drop of saturated NaOH soln. Use 1 ml.

(f) *Thiosulfate soln.*—0.1 N. 24.82 g of  $Na_2S_2O_3 \cdot 5H_2O$  and 3.8 g of  $Na_2B_4O_7 \cdot 10H_2O$  per liter.

Make "blank" determination with each day's run of sugar determinations to guard against changes in the ferricyanide and to make correction for any reducing impurities in the reagents as follows: Combine 5 ml of alcohol, 50.0 ml of the acid buffer soln, and 2 ml of the Na tungstate. To 5 ml of this mixture (used in place of 5 ml of flour extract) add 10.00 ml of the ferricyanide soln and proceed as directed for reducing sugars. (10.00 ml of the thiosulfate should discharge the blue starch-iodine color.) If the titration falls within  $30 \pm .05$  ml do not discard the reagents but make correction in subsequent sugar calculations by using the thiosulfate equivalent of 10 ml of ferricyanide (i.e., ml of the thiosulfate required in above titration) instead of 10.00 as basis for subtraction.

## 20

## DETERMINATION

(a) *Preparation of extract.*—Introduce 5.675 g of flour into 100 or 125 ml Erlenmeyer flask. Tip flask so that all the flour is at one side, then wet flour with 5 ml of alcohol.

Tip flask so that the wet flour is at the upper side and add 50.0 ml of the acid buffer soln, keeping soln from coming in contact with the flour until all has been added to the flask. Then shake flask to bring flour into suspension. Add immediately 2 ml of the Na tungstate soln and again mix thoroly. Filter at once (Whatman No. 4 or equivalent), discarding first 8–10 drops of filtrate.

(b) *Reducing sugars*.—Pipet 5 ml of the flour extract into a test tube of ca 75 ml capacity (Pyrex 1"×8"). Add to test tube exactly 10 ml of the ferricyanide soln, mix, and immerse test tube in a vigorously boiling water bath (surface of liquid in test tube should be 3–4 cm below surface of boiling H<sub>2</sub>O).

21

0.1 N Ferricyanide Maltose-Sucrose Conversion Table\*

0.1 N FERRICYANIDE REDUCED	MALTOSE PER 10 G OF FLOUR	SUCROSE PER 10 G OF FLOUR	0.1 N FERRICYANIDE REDUCED	MALTOSE PER 10 G OF FLOUR	SUCROSE PER 10 G OF FLOUR
ml	mg	mg	ml	mg	mg
0.10	5	5	4.50	237	214
0.20	10	10	4.60	244	218
0.30	15	15	4.70	251	223
0.40	20	19	4.80	257	228
0.50	25	24	4.90	264	233
0.60	31	29	5.00	270	238
0.70	36	34	5.10	276	242
0.80	41	38	5.20	282	247
0.90	46	43	5.30	288	251
1.00	51	48	5.40	295	256
1.10	56	52	5.50	302	261
1.20	60	57	5.60	308	266
1.30	65	62	5.70	315	270
1.40	71	67	5.80	322	275
1.50	76	71	5.90	328	280
1.60	80	76	6.00	334	285
1.70	85	81	6.10	341	290
1.80	90	86	6.20	347	294
1.90	96	91	6.30	353	299
2.00	101	95	6.40	360	304
2.10	106	100	6.50	367	309
2.20	111	104	6.60	373	313
2.30	116	109	6.70	379	318
2.40	121	114	6.80	385	323
2.50	126	119	6.90	392	328
2.60	130	123	7.00	398	333
2.70	135	128	7.10	406	337
2.80	140	133	7.20	412	342
2.90	145	138	7.30	418	347
3.00	151	143	7.40	425	352
3.10	156	148	7.50	431	357
3.20	161	152	7.60	438	362
3.30	166	157	7.70	445	367
3.40	171	161	7.80	451	372
3.50	176	166	7.90	458	377
3.60	182	171	8.00	465	382
3.70	188	176	8.10	472	387
3.80	195	181	8.20	478	392
3.90	201	185	8.30	485	397
4.00	207	190	8.40	492	402
4.10	213	195	8.50	499	407
4.20	218	200	8.60	505	—
4.30	225	204	8.70	512	—
4.40	231	209	8.80	519	—

\* These values are arbitrarily given for 10 g of flour altho the determination is made on only 0.5 g of flour.

After test tube has been in boiling water bath exactly 20 min., cool tube and contents under running  $H_2O$ , and pour at once into a 100 or 125 ml Erlenmeyer flask. Rinse out test tube with 25 ml of the acetic acid-salt soln, add to contents of Erlenmeyer flask, and mix thoroly. Then add 1 ml of the starch-KI soln. Titrate with the  $Na_2S_2O_3$  soln to complete disappearance of blue color (10 ml micro-buret recommended). Subtract the ml of 0.1  $N$  thiosulfate used in titration from 10.00. In case there is a slight blank in the ferricyanide-thiosulfate titration make correction by subtracting from the thiosulfate equivalent of the ferricyanide, 19(f). This difference represents a definite quantity of reducing sugar per 10 g of flour which may be ascertained (as maltose) by consulting the table, 21.

(c) *Non-reducing sugars*.—Pipet 5 ml of the flour extract into an 8" test tube and immerse in vigorously boiling water bath. After boiling 15 min. cool test tube and contents under running  $H_2O$  and add exactly 10 ml of the ferricyanide soln. Proceed as directed under (b). Ferricyanide reduced after hydrolysis—ferricyanide reduced by the maltose in the flour = non-reducing sugars calculated as sucrose and obtained from the table, 21.

22

## PROTEIN—OFFICIAL

Determine  $N$  as directed under II, 21, 22, or 23, and multiply percentage of  $N$  by 5.7 to obtain percentage of protein. Use factor 5.7 to convert  $N$  to protein in wheat used either for manufacturing purposes or for human food.

## 70 PER CENT ALCOHOL-SOLUBLE PROTEINS

23

*By Nitrogen Determination—Tentative*

Transfer 4 g of flour to 150–200 ml bottle or Erlenmeyer flask and add 100 ml of alcohol, 70% by volume, taking care that none of material adheres to bottom of container. Shake thoroly 10–12 times at intervals of 30 min. at room temp., or shake continuously in shaking machine 1 hour, and set aside overnight. Again shake thoroly and filter thru dry, folded filter, returning first runnings to filter until clear filtrate is obtained. Pipet 50 ml of filtrate, equivalent to 2 g of sample, into Kjeldahl flask; dilute with 100 ml of  $H_2O$  to prevent frothing during digestion; and determine  $N$  as directed under II, 21, 22, or 23. Make blank determination on reagents.

*By Polarization—Tentative*

24

## REAGENT

*Millon's reagent*.—Dissolve metallic Hg in equal weight of  $HNO_3$  and dilute soln with equal volume of  $H_2O$ . A freshly prepared soln must be used.

25

## DETERMINATION

Weigh 15.97 g of flour into 300 ml flask and add 100 ml of alcohol (sp. gr. 0.90). Shake at 30 min. intervals 3 hours and let stand overnight. Filter thru dry, folded filter and polarize in 200 mm tube. Precipitate proteins in 50 ml of filtrate by addition of 5 ml of the Millon's reagent. Shake, filter, and polarize filtrate in 200 mm tube. Multiply reading in degrees Ventzke by 1.1 to correct for dilution and deduct product from first reading. This difference  $\times 0.2$  = percentage of gliadin  $N$ .<sup>10</sup>

## 26 PROTEINS SOLUBLE IN 5 PER CENT POTASSIUM SULFATE SOLUTION—TENTATIVE

Weigh 6 g of flour into 200 ml flask and introduce exactly 100 ml of 5%  $K_2SO_4$  soln. Shake at 30 min. intervals 3 hours or, better, agitate at moderate speed in

mechanical shaker 1 hour; let settle 30 min., and filter. Determine N in 50 ml of filtrate as directed under II, 22 or 23, making allowance for N contained in reagents.

27 GLOBULIN AND ALBUMIN<sup>11</sup>(EDESTIN AND LEUCOSIN) AND AMINO NITROGEN—  
TENTATIVE

Weigh 10 g of flour into 500 ml Erlenmeyer flask, add 250 ml of 1% NaCl soln, stopper flask, and shake thoroly. Let stand, with occasional shaking, 3 hours; filter, and evaporate 100 ml of filtrate to small volume in Kjeldahl digestion flask with 5 ml of H<sub>2</sub>SO<sub>4</sub>. Add 25 ml more acid and determine N as directed under II, 21, 22, or 23. To a second 100 ml of filtrate add 5 ml of 20% phosphotungstic acid soln, shake thoroly, allow to settle, and filter by decantation. Wash slightly with H<sub>2</sub>O, concentrate filtrate with 5 ml of H<sub>2</sub>SO<sub>4</sub> in Kjeldahl flask, and determine amino N as directed under II, 21, 22, or 23. Deduct amino N from N found in first fraction to obtain the N as globulin and albumin.<sup>11</sup> Make allowance for N contained in reagents.

GLUTENIN

28

*Method I—Tentative*

Deduct sum of the K<sub>2</sub>SO<sub>4</sub>-soluble N, 26, and the alcohol-soluble N, 23, from total organic and ammoniacal N, 22, and multiply difference by 5.7.

29

*Method II—Tentative<sup>12</sup>*

(Flour and reagents should be allowed minimum exposure to air at all times.)

Weigh 8 g of flour into 200 ml flask, preferably sugar flask or one that readily permits thoro mixing of suspension when shaken. Add 0.2 g of freshly powdered Ba(OH)<sub>2</sub>, follow at once with 50 ml of H<sub>2</sub>O (CO<sub>2</sub>-free), and stopper tightly. Shake immediately to form smooth suspension. Let stand 1 hour at room temp., shaking frequently. Add sufficient 96% methyl alcohol free from acids, aldehydes, and ketones (synthetic methanol preferred) to allow 5 ml of liquid above mark (to correct for volume of flour) when thoroly mixed. Shake vigorously 2 min. After starch settles to bottom, pour supernatant liquid *at once* thru cotton plug, repeating filtrations 2 or 3 times if necessary. Immediately withdraw 50 ml for Kjeldahl N determination, II, 21, 22, or 23. Do not allow more than 15 min. to elapse from time the methyl alcohol is added to withdrawal of the 50 ml aliquot, because gliadin will begin to precipitate after standing short period of time. To prevent troublesome foaming add 150–200 ml of H<sub>2</sub>O to Kjeldahl flask before starting digestion of the alcoholic extract. Convert N to protein by factor 5.7, subtract percentage of protein in extract from percentage of total protein (N×5.7) as determined in separate portion of flour, and record difference as percentage of glutenin in flour.

CRUDE GLUTEN

30

*Qualitative Test<sup>13</sup>—Tentative*

Place small quantity of flour (ca 1.5 mg) on microscope slide; add a drop of H<sub>2</sub>O containing 0.2 g of water-soluble eosin in 1 liter; and mix by means of cover-glass, holding it at first in such a manner that it is raised slightly above slide and taking care that none of flour escapes from beneath it. Finally allow cover-glass to rest on slide and rub it back and forth until gluten has collected into rolls. Carry out operation on white paper so that formation of the gluten rolls can be easily noted. Wheat flour, or other flours containing gluten, show by this treatment a copious quantity of gluten, which absorbs the eosin with avidity, assuming a carmine color.

Rye flour and corn flour yield only trace of gluten; buckwheat flour, no appreciable quantity. If flour is coarse or contains considerable quantity of bran elements, as is true of buckwheat flour and low-grade wheat flour, make test after bolting, as the bran particles and coarse lumps interfere with formation of gluten rolls.

*Quantitative Method—Tentative*

31

(Results are approximate.)

Weigh 25 g of flour into cup or porcelain mortar; add sufficient tap  $H_2O$  (ca 15 ml) to form firm dough ball; and work into dough with spatula or pestle, taking care that none of material adheres to utensil. Allow dough to stand in  $H_2O$  at room temp. an hour; knead gently in stream of tap  $H_2O$  until starch and all soluble matters are removed. Do this operation, which requires ca 12 min., over bolting cloth. To determine whether or not the gluten is starch-free let 1 or 2 drops of the wash  $H_2O$ , obtained by squeezing the gluten, fall into beaker containing perfectly clear  $H_2O$ . If starch is present, cloudiness appears. Allow gluten thus obtained to stand in  $H_2O$  an hour, press as dry as possible with hand, roll into ball, place in weighed flat-bottomed dish, and weigh as moist gluten. Transfer to oven, dry to constant weight at  $100^\circ$  (ca 24 hours), cool, and weigh as dry gluten; or heat moist gluten at ca  $230^\circ$  15–20 min., or until puffed gluten ball has become firm. Dry to constant weight in drying oven.

32 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL<sup>14</sup>—TENTATIVE

Place 20 g of sample in 8 oz nursing bottle, add 100 ml of  $H_2O$  from pipet, shake bottle to prevent lumping of sample, and add exactly 100 ml more  $H_2O$ . Mix contents of stoppered bottle gently by hand or on slowly revolving wheel 1 hour. (Temp. of  $H_2O$  should not exceed  $30^\circ$ .) Centrifuge to facilitate filtration and filter thru thin pad of ignited asbestos (fine) in Hirsch funnel, using light suction. Determine N in 50 ml of filtrate as directed under II, 21, 22, or 23, distilling the  $NH_3$  into 20 ml of 0.1 N acid. Run blank on reagents. Pipet off 100 ml of above filtrate into 200 ml volumetric flask, add 15 ml of NaCl soln (28 g diluted to 300 ml), fill nearly to mark with 95% alcohol, mix carefully to avoid foaming, cool to room temp., make up to mark with alcohol, mix well, and allow to stand overnight. Pipet off supernatant liquid and filter thru  $18\frac{1}{2}$  cm fluted filter paper. Determine N in 100 ml of filtrate as above. (In order to avoid bumping, it is advisable to add the  $H_2SO_4$  and boil off the alcohol before adding the  $K_2SO_4$  and  $HgO$ .) Subtract value obtained from water-soluble N to obtain water-soluble N precipitable by 40% alcohol.

33

LIPOIDS<sup>15</sup>—OFFICIAL

Add 15 ml of alcohol, 70% by volume, to 5 g of flour in 200 ml nursing bottle. Give bottle gentle rotary motion so as to moisten all particles with the alcohol, stopper, and set in water bath kept at  $75$ – $80^\circ$ . Heat 15 min. with frequent mixing by same rotary motion. Add 27 ml of alcohol, stopper bottle, and shake vigorously 2 min. Cool, add 45 ml of ether, and shake well 5 min. (Sample should now be in fine state of division.) Centrifuge just sufficiently to throw solid particles out of suspension but not so as to pack sample too firmly. Decant liquid into 250 ml beaker containing some bits of broken porcelain or glass, and rinse off bottle neck with ether. Re-extract sample with 3 successive 20 ml portions of ether, shake 1 or 2 min. each time, centrifuge, and decant into beaker containing first extract. Evaporate combined ether-alcohol extracts just to dryness on steam bath. Drive off any re-

maintaining moisture on sides of beaker by placing in drying oven at 100° 5 min. Dissolve dry extract in ca 15 ml of  $\text{CHCl}_3$  and filter soln into a previously dried and weighed Pt dish thru pledget of cotton packed in stem of funnel. Free with glass rod any solid extract adhering to beaker and transfer thru filter into first washings by means of  $\text{CHCl}_3$  from wash bottle all extract from beaker bottom and sides. Finally wash funnel and stem tip. Evaporate the  $\text{CHCl}_3$  on steam bath and dry dish and contents in drying oven at 100° to constant weight (75–90 min.). Weigh. Report extract as lipoids.

34

LIPOID PHOSPHORIC ACID<sup>16</sup> ( $\text{P}_2\text{O}_5$ )—OFFICIAL

Dissolve lipoids in 5–10 ml of  $\text{CHCl}_3$ , add 5–10 ml of 4% alcoholic KOH soln, evaporate to dryness on steam bath, and char well in furnace at faint red heat. Cover dish with cover-glass, add sufficient  $\text{HNO}_3$  (1+9) to make soln slightly acid, warm on steam bath, and filter. Wash residue and filter well with hot  $\text{H}_2\text{O}$ . Determine  $\text{P}_2\text{O}_5$  in filtrate as directed under II, 9 or 12. Report as lipid  $\text{P}_2\text{O}_5$ .

UNSAAPONIFIABLE RESIDUE<sup>17</sup>

35

*Modified Kerr-Sorber Method<sup>18</sup>—Tentative*

Place extract from 5 g of flour prepared as directed under 33 in 100–200 ml saponification flask. Add 30 ml of  $\text{C}_2\text{H}_5\text{OH}$  and 3 ml of KOH soln (100 g KOH in 100 ml  $\text{H}_2\text{O}$ ). Place small, short-stemmed funnel in neck of flask to serve as condenser. Boil gently on steam bath ca 20 min., or until complete saponification occurs. Cool to ca 30°, add 50 ml of ether, mix, and transfer to 500 ml separatory funnel (glass stopcock lubricated with water). Rinse the flask with 2 successive 50 ml portions of ether, add to separatory funnel, and mix thoroly. Wash saponification flask with 100 ml of an approximately 0.2 N KOH soln (11.2 g dissolved in 1000 ml of  $\text{H}_2\text{O}$ ) and pour into separatory funnel in slow, steady stream. Rotate funnel very gently to secure better contact of solns but do not shake, because shaking at this stage produces stubborn emulsion. Allow liquids to separate completely and slowly draw off as much of soap soln as possible. Do not draw off any layer of emulsion that may be formed. Keep volume of ether at ca 150 ml by replacing that dissolved by wash solns. Further treat ether soln with 2 successive 100 ml portions of the dilute KOH soln in manner described previously. Test a portion with addition of HCl; if only slightly cloudy soaps are sufficiently removed. Add 30 ml of  $\text{H}_2\text{O}$  to ether and rapidly rotate liquid layers. When layers have separated completely draw off the  $\text{H}_2\text{O}$ , repeating this treatment until washings are free from alkali, as shown by testing with phenolphthalein (3 washings usually suffice). Transfer ether soln quantitatively thru pledget of cotton in stem of funnel to weighed 250 ml Erlenmeyer or beaker flask, containing a few porcelain chips. Before weighing flask dry it and a similar flask or beaker as a counterpoise in oven at ca 100°, and then allow it to stand in air to constant weight. Distil off ether and dry flask and residue at ca 100° to constant weight. Allow flask with unsaponifiable matter and the counterpoise to come to equilibrium with atmosphere before weighing (ca 30 min.). Deduct from weight of unsaponifiable matter any blank obtained from reagents used.

36

## EXTRACT SOLUBLE IN COLD WATER—OFFICIAL

Weigh 20 g of flour into 500 ml Erlenmeyer flask and add gradually 200 ml of  $\text{H}_2\text{O}$  at temp. of ca 0°. Shake vigorously when ca 50 ml of  $\text{H}_2\text{O}$  has been added and continue shaking during addition of remaining  $\text{H}_2\text{O}$ . Allow mixture to stand at 0° 40 min., shaking occasionally. Filter rapidly, returning first runnings to filter, until clear filtrate is obtained. Pipet 20 ml of clear filtrate into weighed dish, evapo-

rate to dryness on steam bath, and dry to constant weight in vacuum oven at ca 100° for periods of 30 min.

STARCH<sup>18</sup>—TENTATIVE

(Applicable to uncooked cereal products.)

37

## REAGENT

*Hydrochloric acid.*—Mix ca equal volumes of HCl and H<sub>2</sub>O and adjust by titration so that 100 ml of this soln contains 20.5–21.0 g of HCl.

38

## DETERMINATION

Weigh accurately sufficient finely ground sample (should readily pass thru 20-mesh sieve) to represent 0.5–1.0 g of starch. Transfer to funnel fitted with 9 cm S & S No. 589 white ribbon or Whatman No. 40 filter paper and extract by nearly filling the filter 4 times with ethyl ether; likewise extract with alcohol (70% by volume) and with H<sub>2</sub>O. Allow to drain 1 hour uncovered. Transfer drained filter and contents to 50 ml beaker. In next step use stirring rod having flattened button-like end 15 mm in diameter, and (very important) tamp with a twisting motion during time specified in order to get filter paper completely disintegrated and thus insure complete suspension of starch in the HCl soln without hydrolysis of any of it. Complete maceration while there is small amount of HCl present and whole contents are a rather thick paste. (If this optimum condition is obtained practically duplicate results will follow.) Add the HCl reagent at 15° to beaker containing sample, using a fast delivering 10 ml Mohr pipet with 1 ml marked off at lower end with heavy pencil marks. Keep acid supply on bench, but do not allow it to get above 18°.

Proceed as follows, adding the HCl in quantities given: Add 1 ml, tamp 1 min.; add 1 ml, tamp 2 min.; add 1 ml, tamp 2 min.; add 1 ml, tamp 1 min.; add 1 ml, tamp 1 min.; add 1 ml, tamp 1 min.; add 1 ml, tamp 1 min.

Fill beaker half full with the acid and stir 30 sec. Fill beaker  $\frac{3}{4}$  full and stir 30 sec. (In 10 min. by this treatment paper should be completely disintegrated and in smooth state of suspension; tamping should be continued vigorously during this time, and as little time as possible should be spent adding the acid.) Immediately transfer to 100 ml wide-mouthed volumetric flask, rinsing out beaker with the HCl; carefully make to volume with the HCl reagent and add 0.5 ml for volume of filter paper (this step requires 2 min.). Shake stoppered flask vigorously 5 min., and allow to stand 5 min. in beaker of H<sub>2</sub>O at 20°. Shake twice and filter immediately into 250 ml suction flask thru small Büchner funnel (41 mm in diameter) fitted with thin layer of asbestos and filled half full with dry, fluffy asbestos. (The filtration requires 1 min. only.) Immediately pipet 50 ml of the filtrate into 200 ml beaker (tall form) containing 115 ml of alcohol. (The quantity of starch finally weighed will then vary from 0.25 to 0.5 g. Time consumed from initial addition of acid is 24 min.) Allow the pipet to drain completely and then stir with whipping motion 1 min. to flocculate precipitated starch. Wash down sides of beaker with 70% alcohol. Allow to stand 3–4 min., until nearly all precipitate has settled, and then carefully decant supernatant liquid, which is somewhat turbid, so that little or no precipitate passes into weighed Gooch crucible, which has been fitted with thin pad of ignited asbestos and is half filled with fluffy ignited asbestos. Wash precipitate and filter by decantation, using successively two 40 ml portions of 70% alcohol (by volume), then 4 times, using ca 30 ml portions of alcohol, each time breaking up precipitate by rapid stirring and allowing precipitate to settle before decantation. After each stirring rinse sides of beaker with small stream of alcohol to prevent starch from drying and



sticking. Finally transfer starch completely by means of jet of alcohol and wash sides of Gooch and precipitate with a little of the alcohol. (All these filtrations are very fast.) Dry crucible and contents uncovered 2 hours at 130°; cover crucible immediately and place in desiccator charged with  $P_2O_5$ , fresh  $H_2SO_4$ , or freshly ignited  $CaO$ ; cool 10 min. and weigh. Multiply result by 2 and report as starch. *Caution:* To obtain satisfactory results, these directions must be followed carefully in every detail. As the steps are timed it is essential to learn procedure so that no time will be lost in following it thru. Arrange everything needed in determination before the  $HCl$  is added to sample.

#### CHLORINE

##### 39 *Qualitative Test (Chlorine-Bleached Flours)—Tentative*

Extract 30 g of flour with 50 ml petroleum benzin and allow solvent to evaporate. (Small quantity of oil remains.) Heat piece of  $Cu$  wire in colorless gas flame until it is black and no longer colors flame green. Dip hot end of wire into oil and again bring into flame. If  $Cl$  or  $Br$  has been used as bleaching agent, green or blue coloration is produced.

#### CHLORINE IN FAT<sup>20</sup>—OFFICIAL, FIRST ACTION

40

##### *Quick Ashing Method*

#### EXTRACTION OF FAT

Weigh 500 g of the flour into 2 liter flask. Add 700 ml of petroleum benzin and shake at 5 min. intervals for 30 min. Filter thru Büchner funnel, pressing flour to obtain as much solvent as practicable. Transfer the benzin extract to large beaker and evaporate on steam bath to ca 10 ml. Filter into container thru small funnel containing pledget of cotton packed firmly in stem. (Filtrate must be clear and free from flour.)

41

#### DETERMINATION

Heat porcelain crucible of ca 90 ml capacity containing 10 g of fusion mixture (138 g of anhydrous  $K_2CO_3$ , 106 g of anhydrous  $Na_2CO_3$ , and 75 g of powdered  $KNO_3$ ) 30 min. in 100° oven; dry in desiccator and weigh. Transfer the filtered 10 ml of benzin extract to the crucible, using petroleum benzin for rinsing. Evaporate off the petroleum benzin on steam bath and dry fat in 100° oven for 30 min. Cool, and determine weight of fat by difference. Add to the crucible 5 g more of the fusion mixture and spread evenly. Burn to a white ash in muffle at temp. 525° (ca 1 hour).

Add 25 ml of hot  $H_2O$  to mixture and transfer with small quantity of hot  $H_2O$  to 200 ml tall-form beaker or beaker flask. Add  $HNO_3$  cautiously until soln is slightly acid to litmus paper. Add 25 ml more  $HNO_3$ . Add 5 ml of 0.3  $N$   $AgNO_3$  soln. Boil 5 min. in hood. Cool to room temp. Filter thru 9 cm No. 1 Whatman filter paper, or similar  $Cl$ -free filter paper. Use 1%  $HNO_3$  soln for rinsing. Proceed with the digestion as directed under XII, 39, beginning, "Place filter paper and contents in Kjeldahl flask." After digestion use 175 ml of  $H_2O$ . Run a blank on the reagents. Report  $Cl$  as mg per 1 g of fat.

#### NITRITE NITROGEN—TENTATIVE

42

#### REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 0.5 g of sulfanilic acid in 150 ml of 20% acetic acid.

(b) *Alpha-naphthylamine hydrochloride soln.*—Dissolve, by heating, 0.2 g of the salt in 150 ml of 20% acetic acid.

(c) *Standard nitrite soln.*—Dissolve 0.1097 g of dry  $\text{AgNO}_2$  in ca 20 ml of hot  $\text{H}_2\text{O}$ , add 0.10 g of  $\text{NaCl}$ , shake until the  $\text{AgCl}$  flocculates, and dilute to 1 liter. Draw off 10 ml of the clear soln and dilute to 1 liter. 1 ml of this nitrite soln = 0.0001 mg of N.

The  $\text{AgNO}_2$  may be prepared as follows: To cold soln of ca 2 g of  $\text{NaNO}_2$  or  $\text{KNO}_2$  in 50 ml of  $\text{H}_2\text{O}$ , add soln of  $\text{AgNO}_3$  so long as precipitate forms. Decant liquid and thoroly wash precipitate with cold  $\text{H}_2\text{O}$ . Dissolve in boiling  $\text{H}_2\text{O}$ . (On cooling, the  $\text{AgNO}_2$  crystallizes out.) Dry crystals in dark at ordinary temp. (preferably in vacuum).

## 43

## DETERMINATION

(1) Select a series of 100 ml volumetric flasks of uniform dimensions and color and place 2 g of high-grade, nitrite-free flour in each flask; add ca 70 ml of nitrite-free  $\text{H}_2\text{O}$  and shake until flour is thoroly moistened. Add to flasks varying quantities of the standard  $\text{NaNO}_2$  soln, so that a series of comparison standards will be obtained having a range covering probable nitrite content of unknown sample. Reserve one flask for blank test. In order to avoid making a large series of standards it is well to make preliminary test to ascertain approximate nitrite content of unknown. If quantity of nitrite present is small, the nitrite soln in flasks may be increased by 0.4 ml each. If bleaching is excessive, 1 g of flour may be used thruout, or standards may be given a wider variation in nitrite content.

To each of 2 similar flasks add 2 g of the flour and 90 ml of  $\text{H}_2\text{O}$ , shake thoroly, digest all the flasks, including blank, in water bath at  $40^\circ$  at least 15 min., and add 2 ml each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns to each flask, shaking mixture after addition of each reagent. Continue digestion at  $40^\circ$  for an additional 20 min. (The color must be developed in all the flasks under conditions as nearly uniform as possible.) Make up to marks with nitrite-free  $\text{H}_2\text{O}$  and compare unknown with series of standards. (This may be done in large, white-enameled pan, effect of turbidity due to flour being minimized by white background.) Solns should be allowed to subside and should not be shaken during comparison. Or,

(2) Weigh 20 g of the flour into 500 ml Erlenmeyer flask; add 200 ml of nitrite-free  $\text{H}_2\text{O}$ , previously warmed to  $40^\circ$ ; and close flask with rubber stopper. Shake vigorously 5 min. and digest 1 hour in water bath, keeping temp. of liquid in flask at  $40^\circ$  and shaking at 10 min. intervals. Finally filter thru a nitrite-free filter. Return first runnings to filter until clear filtrate is obtained. Pipet 50 ml of filtrate and 50 ml of standard nitrite soln into small flasks; add to each 50 ml of  $\text{H}_2\text{O}$  and 2 ml each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns; shake; and allow to stand 1 hour to bring out color. Compare two solns in colorimeter. Divide height of column of standard soln by that of the soln of sample to obtain parts of nitrite N (free and combined) per million of flour.

## 44

BENZOYL PEROXIDE BLEACH IN FLOUR<sup>21</sup>—TENTATIVE

Add a mixture of 125 g of flour, 100 g of salt, and 80 g of  $\text{CaCl}_2$  (dried) to short-necked 800 ml Kjeldahl flask that contains 250 ml of  $\text{H}_2\text{O}$  and 30 ml of  $\text{HCl}$ , and shake vigorously. Immediately connect for steam distillation and distil 325 ml as rapidly as possible after initial foaming. Saturate distillate with 100 g of salt, transfer to 500 ml separatory funnel, and extract with 50 ml of ethyl ether. Again extract salt soln with 50 ml of ethyl ether and discard salt soln. Pour ether from two funnels into large, flat crystallizing dish and evaporate at room temp. with aid of electric fan. Dissolve residue in 5 ml of acetone, add 7 ml of 2 N  $\text{NaOH}$ , and transfer to 150 ml beaker. Rinse crystallizing dish into beaker with 35–40 ml of  $\text{H}_2\text{O}$ . Heat

over flame, carefully at first, then boil ca 20 min. until all acetone is removed. Add  $\text{H}_2\text{O}$  occasionally to keep volume ca constant. While hot transfer to separatory funnel and add 25 ml of amyl alcohol. Pour lower layer into 250 ml separatory funnel and extract again with 20 ml of amyl alcohol. Pour lower layer into 250 ml casserole. Combine amyl alcohol solns, add equal volume of petroleum benzin, extract 3 times with 5 ml of  $\text{H}_2\text{O}$ , and add to aqueous soln in casserole. Discard the amyl alcohol-benzin soln. Add 2 ml of superoxol (30%  $\text{H}_2\text{O}_2$ ) to aqueous soln. Bring to boiling slowly and boil until foaming ceases. Cool. Make acid to litmus with  $\text{H}_2\text{SO}_4$  (1+1). Pour into small separator. Cool, and extract twice with 20 ml of mixture of equal parts of ethyl ether and petroleum benzin. Pour combined extracts into large test tube, add 2 ml of 2 N NaOH, stopper tube, and shake. Place thread in tube to insure even boiling and evaporate slowly at first, by holding over steam. Place tube in vigorously boiling saturated salt soln. Add drop of superoxol and when foaming ceases add another drop. Continue adding a drop at a time until soln is almost colorless. Add a drop or two of  $\text{H}_2\text{O}$  occasionally if evaporation is too rapid. Evaporate completely to dryness and heat at  $100^\circ$  in vacuum ca 30 min. Cool, and add 0.3 g of  $\text{KNO}_3$  and 3 ml of  $\text{H}_2\text{SO}_4$ . Heat in boiling water bath 20 min., taking care to get all solid material into soln (stirring rod is essential). Cool the tube in cold  $\text{H}_2\text{O}$  and add 6 ml of  $\text{H}_2\text{O}$  with stirring. When cool, add 15 ml of  $\text{NH}_4\text{OH}$  slowly with continuous stirring to keep soln cool. Add 2 ml of 6% hydroxylamine hydrochloride soln and place in  $65^\circ$  water bath 5-6 min., stirring occasionally. Cool in cold  $\text{H}_2\text{O}$ , filter into another similar tube, and observe color of filtrate. A red color indicates presence of benzoic acid.

To make this method semiquantitative proceed as follows:

Prepare series of standard tubes containing 0.2-1.5 mg of benzoic acid in ether soln (1 mg to 1 ml). Add 2 ml of 2 N NaOH to each. Mix by shaking and proceed exactly as with sample, starting, "place tube in vigorously boiling saturated salt soln." Comparison of sample with standards familiarizes the analyst with color to be expected and offers approximate estimation of amount of benzoic acid recovered. For calculation of approximate amount of benzoic acid in p.p.m. multiply sample reading in mg by 32. This factor is based on 125 g sample and minimum recovery of 25% benzoic acid.

#### 45

#### GASOLINE COLOR VALUE—TENTATIVE

Place 20 g of flour in wide-mouthed, glass-stoppered 120 ml bottle and add 100 ml of colorless gasoline. Stopper tightly and shake vigorously 5 min. Allow to stand 16 hours, shake again a few seconds until flour has been loosened from bottom of bottle and thoroly mixed with gasoline, then filter immediately thru dry 11 cm paper into Erlenmeyer flask, keeping funnel covered with watch-glass to prevent evaporation. In order to secure clear filtrate, allow a certain quantity of the flour to pass over into filter, and pass first portion of filtrate thru a second time. (It will be found convenient to fit filter paper to funnel by means of  $\text{H}_2\text{O}$  and to dry thoroly either by standing overnight in well-ventilated place or by heating.)

Determine color value of clear gasoline soln in Schreiner or similar colorimeter, using for comparison 0.005%  $\text{K}_2\text{CrO}_4$  soln. This soln corresponds to a gasoline number of 1.0 and is conveniently prepared by diluting 10 ml of a 0.5% soln to 1 liter. Adjust colorimeter tube containing gasoline soln to read 50 mm, and raise or lower tube containing standard chromate soln until shades of yellow in both tubes match. Reading of chromate soln  $\div$  reading of gasoline soln = gasoline color value. Color value may also be determined in Nessler tubes by using for comparison  $\text{K}_2\text{CrO}_4$  solns of various dilutions prepared from 0.5% soln and filling tubes in all cases to height of 50 mm.

DETECTION OF RYE FLOUR IN WHEAT FLOUR<sup>22</sup>

46

*Chloroform Test—Tentative*

(a) *In ordinary flours.*—To 10 g of flour in test tube, add 20 ml of  $\text{CHCl}_3$ , stopper tube, and shake well. Allow tube to stand in vertical position until heavier particles have settled out, preferably overnight. If rye is present, sediment in tube will be of greenish or bluish tint. Wheat gives yellowish sediment.

Make comparisons with wheat and rye flours of known purity and with mixtures of varying proportions, such as 5, 10 and 15%, etc., of rye.

(b) *In phosphated flours.*—Treat flours containing phosphate or leavening agents with  $\text{CCl}_4$  in separatory funnel to remove added salts. After removing sediment of salts from the separatory funnel collect flour on filter, transfer to test tube, and treat with  $\text{CHCl}_3$ .

DIASTATIC ACTIVITY OF FLOUR<sup>23</sup>—OFFICIAL

47

## REAGENTS

(a) *Buffer soln.*—Make up 3 ml of glacial acetic acid and 4.1 g of *anhydrous* sodium acetate to 1 liter with  $\text{H}_2\text{O}$ . The pH of this soln is 4.6–4.8.

(b) *Alkaline ferricyanide soln.*—16.5 g of pure dry  $\text{K}_3\text{Fe}(\text{CN})_6$  and 22 g of anhydrous  $\text{Na}_2\text{CO}_3$  in 1 liter of  $\text{H}_2\text{O}$ . The  $\text{K}_3\text{Fe}(\text{CN})_6$  soln is 0.05 N. It maintains its strength for long period of time if kept in dark glass bottle away from light. (The best C. P. grade of this salt purchased on market may ordinarily be depended upon to be free from moisture and impurities.)

(c) *Sodium thiosulfate soln.*—0.05 N. 12.41 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  per liter. Select only clear crystals from best C.P. grade. If redistilled  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  (second distillation being made after addition of small quantity of alkaline K permanganate to first distillation, to destroy all traces of organic matter) is used in making up this soln, it will retain its normality a long time, whereas with ordinary distilled  $\text{H}_2\text{O}$  it is likely to deteriorate slowly on standing. Check the ferricyanide against the thiosulfate soln as follows: To 10 ml of the ferricyanide soln add 25 ml of the acetic acid reagent (d) followed by 1 ml of 50% KI and 2 ml of soluble starch soln. Titrate with the Na thiosulfate soln. (It should require exactly 10 ml of the Na thiosulfate to completely discharge blue starch-iodine color.) Standardize the Na thiosulfate soln against pure I soln if necessary.

(d) *Acetic acid soln.*—200 ml of glacial acetic acid, 70 g of KCl, and 20 g of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  per liter.

(e) *Potassium iodide soln.*—50% soln of KI. Add 1 drop of NaOH (1+1) for each 100 ml of soln to prevent or substantially delay deterioration of the soln (with liberation of I) on standing, which will otherwise occur. (Soln must be colorless.)

(f) *Soluble starch soln.*—1% of soluble starch in 30% NaCl soln. Prepare soluble starch suspension and pour slowly into boiling  $\text{H}_2\text{O}$ . Add salt and make to volume. (Soln should be transparent and colorless.)

48

## DETERMINATION

(Total maltose after diastasis for 1 hour.)

Introduce 5 g of flour and teaspoonful of ignited quartz sand into 100 or 125 ml Erlenmeyer flask, and mix by rotating flask. Add 46 ml of buffer soln, and again mix by rotating flask until all flour is in suspension. (Flask and all ingredients should be *individually* brought to 30° before being mixed together.) Digest 1 hour at 30°, preferably in an accurately controlled water thermostat, shaking flask (by rotation)



every 15 min. At end of the hour add 2 ml of  $\text{H}_2\text{SO}_4$  ( $3.58 \pm 0.05 N$ , ca 1+9), and mix thoroly. Add 2 ml of 12% Na tungstate soln, mix, and let stand 1-2 min. Filter thru paper (No. 4 Whatman or its equivalent), discarding first 8 or 10 drops, and pipet 5 ml of filtered extract into test tube of ca 50 ml capacity (18-20 mm diameter). Pipet exactly 10 ml of the ferricyanide soln into the 5 ml of extract in test tube, and immerse test tube in vigorously boiling water bath. Have surface of liquid in test tube 3-4 cm below surface of boiling  $\text{H}_2\text{O}$ . (The delay between filtering of extract and treatment in boiling water bath should not be more than 15-20 min. Further delay may cause slight error due to sucrose hydrolysis in the acid soln.) Allow test tube to remain in boiling water bath *exactly* 20 min. Cool test tube and its contents under running  $\text{H}_2\text{O}$ , and pour at once into 100 or 125 ml Erlenmeyer flask. Rinse out test tube with 25 ml of the acetic acid soln, and add to contents of Erlenmeyer flask, with thoro mixing. Add 1 ml of the KI soln followed by 2 ml of the starch soln, and mix thoroly. Titrate with 0.05  $N$  Na thiosulfate to the complete disappearance of blue color (10 ml buret is recommended). Subtract number of ml of 0.05  $N$  Na thiosulfate used in titration from 10, which gives ml of 0.05  $N$  ferricyanide reduced to ferrocyanide by reducing sugars in flour extract. This value represents a definite quantity of maltose, which may be ascertained by consulting table (49). When 5 ml of flour extract is used, as herein specified, it is necessary merely to multiply mg of maltose by 20 to obtain mg of maltose per 10 g of flour in 1 hour's diastasis. This is the value that is recorded and reported as measure of diastatic value of flour in question.

The foregoing specifications may be used with all ordinary flours whose values for

49

*Maltose conversion table\**

0.05 $N$ FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 $N$ FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 $N$ FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 $N$ FERRI- CYANIDE REDUCED	MALTOSE EQUIVALENT
ml	mg	ml	mg	ml	mg	ml	mg
0.1	0.2	2.6	4.2	5.1	8.3	7.6	12.3
0.2	0.3	2.7	4.4	5.2	8.4	7.7	12.5
0.3	0.5	2.8	4.5	5.3	8.6	7.8	12.7
0.4	0.6	2.9	4.7	5.4	8.7	7.9	12.9
0.5	0.8	3.0	4.9	5.5	8.9	8.0	13.0
0.6	1.0	3.1	5.0	5.6	9.1	8.1	13.2
0.7	1.1	3.2	5.2	5.7	9.2	8.2	13.4
0.8	1.3	3.3	5.3	5.8	9.4	8.3	13.5
0.9	1.5	3.4	5.5	5.9	9.6	8.4	13.7
1.0	1.6	3.5	5.7	6.0	9.7	8.5	13.9
1.1	1.8	3.6	5.8	6.1	9.9	8.6	14.0
1.2	1.9	3.7	6.0	6.2	10.0	8.7	14.2
1.3	2.1	3.8	6.2	6.3	10.2	8.8	14.4
1.4	2.3	3.9	6.3	6.4	10.4	8.9	14.6
1.5	2.4	4.0	6.5	6.5	10.5	9.0	14.8
1.6	2.6	4.1	6.6	6.6	10.7	9.1	15.0
1.7	2.8	4.2	6.8	6.7	10.9	9.2	15.2
1.8	2.9	4.3	7.0	6.8	11.0	9.3	15.4
1.9	3.1	4.4	7.1	6.9	11.2	9.4	15.6
2.0	3.2	4.5	7.3	7.0	11.3	9.5	15.9
2.1	3.4	4.6	7.5	7.1	11.5	9.6	16.1
2.2	3.6	4.7	7.6	7.2	11.7	9.7	16.5
2.3	3.7	4.8	7.8	7.3	11.8	9.8	17.0
2.4	3.9	4.9	7.9	7.4	12.0	9.9	—
2.5	4.1	5.0	8.1	7.5	12.2	10.0	—

\* Prepared by applying the specified procedure to standard solns of pure maltose and using all reagents in the quantities and volumes precisely as used for flour extracts.

mg of maltose produced by 10 g of flour in 1 hour will seldom, if ever, exceed 350. For material giving higher values, such as products from malted or sprouted grain, use smaller portions of extract, *i.e.*, 1, 2, or 3 ml instead of 5 ml. In such cases, however, add enough distilled  $H_2O$  to make up difference, and use different factor for converting results into mg of maltose per 10 g of flour. Thus, when 2 ml of extract is used, multiply value obtained from table by 50 instead of 20. If material in test tubes is colorless instead of yellow, after treatment in the boiling water bath, and gives no blue color upon addition of KI, it is apparent that there was more than enough maltose to reduce all the ferricyanide, and that determination must be repeated with a smaller quantity of extract.

50

## BLANK DETERMINATION

A blank determination, designed to indicate quantity of reducing sugar originally present in the flour—value for which presumably should be deducted from total maltose value after 1 hour's diastasis—has been generally regarded as an essential step in the estimation of flour diastatic activity. This operation, however, is ordinarily unnecessary when dealing with flour milled from *sound* wheat, because quantity of reducing sugars originally present as such is so small and so nearly constant that it may be disregarded for all practical purposes. The blank determination may therefore be conveniently omitted in ordinary routine testing. It need be used only when there is occasion to doubt soundness of the wheat, or in cases where there is known to have been an appreciable quantity of frosted, sprouted, heat-damaged, or otherwise unsound kernels in the wheat from which the flour was milled.

To make blank determination, proceed as follows: Add to 5 g of flour and a teaspoonful of quartz sand in 100 or 125 ml Erlenmeyer flask 48 ml of 0.4% (by volume)  $H_2SO_4$  (preferably pre-cooled to ice-water temp.). Shake mixture thoroly again, allow to stand 2 min. and filter thru No. 4 Whatman (or its equivalent) paper. Using 5 ml of clear filtrate, proceed according to 48.

## APPARENT VISCOSITY OF ACIDULATED FLOUR-WATER SUSPENSION

*By MacMichael Viscosimeter<sup>24</sup>—Official*

51

## ADJUSTMENT OF MACHINE

- (a) Use a No. 30 MacMichael viscosimeter wire.
- (b) Have diameter of disk plunger 2.375",  $\pm 0.01$ ".
- (c) Adjust machine so that clearance between bottom of disk and inner surface of bottom of bowl is 0.25",  $\pm 0.005$ ". Check this clearance carefully with depth gage reading in 0.001".
- (d) Use a viscosimeter bowl having a diameter of ca 7 cm (depth of bowl will vary according to age of machine).
- (e) Adjust regulating device to permit a speed of exactly 12 r.p.m., and check it carefully and frequently with a stop-watch, because as motor warms up machine will have tendency to increase its speed.
- (f) Adjust machine and keep it level, and when bob is placed see that it is riding freely and not touching sides of guide.
- (g) Adjust dial so that when it comes to rest pointer is on zero mark.

52

## PREPARATION OF LACTIC ACID

Add to concentrated lactic acid approximately the right proportion of  $H_2O$  to give a slightly stronger soln than normal. Reflux this soln 3 hours, cool, and by addition of  $H_2O$  adjust to normal. Or proceed as follows: Use enough concentrated

lactic acid to prepare a soln ca 0.8500 *N* when standardized with 0.1 *N* NaOH. Transfer this soln to an Erlenmeyer flask fitted with air condenser to prevent undue evaporation of H<sub>2</sub>O, and heat at temp. of 80° for 24 hours (soln will have increased in strength to 1.183 *N*). Add H<sub>2</sub>O to bring the soln to exactly normal.

53

## PREPARATION OF FLOUR-WATER SUSPENSION

Into a clean, dry, 500 ml Erlenmeyer flask, place 20 g of flour on 15% moisture basis and add 100 ml of H<sub>2</sub>O at 30°. Place rubber stopper in mouth of flask and shake vigorously 1 min. Place flask in constant temp. cabinet or water bath at 30° for 1 hour, shaking ca 10 times every 15 min. Remove flask from cabinet or water bath, add 3 or 4 drops of caprylic alcohol, shake 10 times to remove any foam that may be present, and pour suspension in bowl of viscosimeter.

54

## DETERMINATION

After pouring the suspension into viscosimeter bowl, make sure that bowl is down flush on its supports. Start machine, but before placing the bob or disk in place, stir soln with bob 25 times to insure uniform suspension. Place the wire of bob in its holder and take reading after damping swing of dial by placing a finger on indicator pointer and then gradually touching swinging dial. Make second reading after addition of 1 ml of normal lactic acid, and likewise the third and following readings after addition of 2 ml increments of the normal lactic acid. Do not stop motor between readings. After or during the addition of lactic acid, stir suspension 25 times by up-and-down motion of bob. Suspend bob by the wire and take reading. Determine maximum apparent viscosity of the acidulated flour-H<sub>2</sub>O suspension by plotting apparent viscosity readings against volume of acid added. Usually a total of 7 ml of 1 *N* lactic acid is sufficient to give maximum reading, but 2 ml increments should be added continuously until no further increase in apparent viscosity is noted.

SOYA FLOUR IN UNCOOKED CEREAL PRODUCTS<sup>25</sup>

55

*Qualitative Test*

Place ca 0.5 g of sample in small test tube containing a strip of red litmus paper partly immersed in 5 ml of 2% soln of urea. Mix, stopper tube, and heat at 40° for 3 hours. If soya bean flour is present in more than traces the litmus will be colored blue. (Bromothymol blue may also be used as an indicator, which likewise turns blue if soya bean flour is present.)

RYE, OATS, CORN, BUCKWHEAT AND THEIR  
PRODUCTS—TENTATIVE

56

MOISTURE.—*See* 2.

57

ASH.—*See* 5.

58

FAT.—*See* XXVII, 22.

59

CRUDE FIBER.—*See* XXVII, 25-27.

60

PROTEIN (*N* × 6.25).—*See* II, 21, 22, or 23.

## BAKED CEREAL PRODUCTS

## BREAD

61

PREPARATION OF SAMPLE<sup>26</sup>—OFFICIAL

(To be used when total solids of original entire loaf is not desired.)

Cut loaf, or  $\frac{1}{2}$  loaf, of bread into slices 2-3 mm thick. Spread slices on paper and

allow to dry in warm room until sufficiently crisp and brittle to grind well in a mill. Grind entire sample to pass 20-mesh sieve, mix well, and keep in air-tight container.

62

TOTAL SOLIDS IN ENTIRE LOAF OF BREAD<sup>26</sup>—OFFICIAL

Accurately weigh loaf of bread immediately upon receipt (*A*), using scales sensitive to at least 0.2 g. Should accurate weighing be impossible at this time, seal sample in air-tight container and accurately weigh as soon thereafter as is practicable (*A*). Preserve sample in such manner that no loss of bread solids can occur, whereby loss would be calculated as moisture. Cut bread into slices 2–3 mm thick ( $\frac{1}{3}$  of loaf may be used). Spread slices on paper, allow them to dry in warm room (ca 15–20 hours), and when apparently dry, break into fragments. If bread is not entirely crisp and brittle, allow it to dry longer—until it is in equilibrium with moisture of air—in order that no moisture changes may occur during grinding. Quantitatively transfer air-dried bread to scale pan and accurately weigh (*B*). Grind sample just to pass a 20-mesh sieve, mix well, and keep in air-tight container. Determine percentage of total solids (*C*) of ground sample as directed under 3 or 4. Calculate total solids of bread from the formula:—

$$T.S. = \frac{\frac{B \times C}{100} \times 100}{A}, \text{ or } \frac{B \times C}{A}, \text{ in which}$$

*A* = weight of loaf (or  $\frac{1}{3}$  loaf) at time of receipt;

*B* = weight of the air-dried sliced bread; and

*C* = percentage of total solids in prepared ground sample.

TOTAL SOLIDS OF AIR-DRIED GROUND SAMPLE<sup>26</sup>

63

*Method I—Official*

Use 2 g of prepared sample, 61, and proceed as directed under 3.

64

*Method II—Official—See 4.*

65

FAT AND FAT NUMBER<sup>27</sup>

Slice one loaf of bread, place in wire rack, and allow to dry overnight, or until sufficiently dry to grind. Grind bread to ca size of openings in 20-mesh sieve, mix, sample, and transfer 50 g to 600 ml beaker. Add 100 ml of H<sub>2</sub>O and mix. Add 100 ml of HCl, mix, cover, and keep on steam bath 1 hour, stirring well 6 or 7 times. Cool in cold (15° or less) water bath, add 50 ml of ice-cold H<sub>2</sub>O, and stir. Add 10 g of filter cel, stir, and mix in completely. Prepare 90 mm Büchner funnel as follows:

Place two No. 590 S & S 9 cm filter papers in funnel and apply suction. Mix 10 g of filter cel with 50 ml of H<sub>2</sub>O and rapidly pour mixture into funnel. (This should make a smooth even layer of the filter cel over the whole filter paper, with no crack or opening.) Filter sample immediately. Rinse out beaker several times with ice-cold H<sub>2</sub>O. Just before filtration is complete, wash down sides of Büchner with ca 100 ml of ice-cold H<sub>2</sub>O. Up to this point do not allow pad to suck dry. Continue with suction until filter cel pad seems dry. Transfer this mass, without filter paper, from Büchner to original beaker. Rinse off filter paper and funnel with petroleum benzin and add benzin to beaker containing the dry mass. Break up mass with a rod, and dry overnight on steam bath to remove the H<sub>2</sub>O. Heat in oven at 100° ca 1 hour to remove all moisture (material must be dry or fat results will be low). Add 25 g of anhydrous



$\text{Na}_2\text{SO}_4$  and break up any lumps. Prepare large Knorr extraction tube of ca 200 ml capacity (glass tubing 5 cm in diameter with height of 12 cm from shoulder to top of tube). Pack tube with asbestos tamped tightly to form pad ca  $\frac{3}{8}$ " thick. Insert stem of tube into 2-holed rubber stopper in filtering bell jar connected to suction thru 2-way stopcock. Place 500 ml Erlenmeyer flask within bell jar so that stem of tube passes thru neck of flask. To cool beaker and contents add 150 ml of mixed ethyl ether and petroleum benzin, equal parts of each, and macerate against sides of beaker with medium sized stiff metal spatula 3–4 min. Decant onto extraction tube. Add to beaker 80 ml of the mixed ether. Work as before 2 min., likewise decant. Transfer contents of beaker to extraction tube, suck dry, and tamp with flattened stirring rod until all ether is removed. To material in tube add 100 ml of the mixed ethers that have just previously been used to rinse out beaker, mix thoroly with stirring rod a few minutes, allow to stand a minute, then suck dry, and tamp material as before. Likewise make two additional extractions, turning suction on and off carefully to avoid loss of sample in Erlenmeyer flask. Hang thread in flask from top so that it touches the bottom. (Time may be saved by transferring to tall-form 1 liter beaker.) Evaporate on steam bath, completely transfer fat with small amounts of petroleum benzin to tared 150 ml beaker, carefully evaporate benzin on steam bath, dry at  $100^\circ$  to constant weight (ca 30 min.), cool, and weigh. Figure percentage of total fat on moisture-free basis.

Weigh duplicate samples of 1 g (within  $\pm .03$  g) of fat into 300 ml Florence flasks, add 4 ml of glycerol-soda soln, **XXXI, 28(c)**, and saponify as directed under **XXXI, 29**. Cool, add few pieces of pumice stone previously ignited, 138 ml of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , and 3 ml of  $\text{H}_2\text{SO}_4$  (1+4), and proceed as directed in **XXXI, 29**, using same apparatus. Use 0.02 *N* NaOH for titration and report number of ml per 1 g of fat. Multiply ml of 0.02 *N* NaOH used by 1.1 and divide by weight of fat taken to obtain "fat number." Run blank determination.

**66****CITRIC ACID<sup>28</sup>—TENTATIVE**

To a weight of air-dried bread equivalent to 77.7 g of moisture-free bread in 500 ml volumetric flask, add 400 ml of mixture containing 25 ml *N*  $\text{H}_2\text{SO}_4$ , 20 ml of 20% phosphotungstic acid soln, 55 ml of  $\text{H}_2\text{O}$ , and sufficient 95% alcohol to make 500 ml. Shake 5 min., make to mark, and allow to stand overnight. Readjust to mark with 95% alcohol, shake 5 min., and filter with suction on paper in 12 cm Büchner funnel. Transfer 325 ml of filtrate to centrifuge bottle, add 30 ml of Pb acetate soln (75 g of the salt +1 ml of glacial acetic acid diluted to 250 ml with  $\text{H}_2\text{O}$ ), shake 5 min., and centrifuge at ca 900 r.p.m. 15 min. Decant supernatant liquid (disregard turbidity), allow to drain, transfer residue with ca 150 ml of  $\text{H}_2\text{O}$  to 250 ml volumetric flask, and thoroly saturate with  $\text{H}_2\text{S}$ . Make to mark with  $\text{H}_2\text{O}$ , shake thoroly, and filter thru large folded filter. Evaporate 200 ml of clear filtrate in 500 ml Erlenmeyer flask over free flame to ca 75 ml. Cool to  $45\text{--}50^\circ$ ; add 10 ml of  $\text{H}_2\text{SO}_4$  (1+1), 5 ml of KBr soln (15 g in 40 ml of  $\text{H}_2\text{O}$ ), and 15 ml of permanganate soln (5 g of  $\text{KMnO}_4$  diluted to 100 ml). After ca 2 min., stopper Erlenmeyer, shake vigorously, and allow to stand 3 min. longer. Add 20 ml of  $\text{FeSO}_4$  soln (40 g of the salt +1 ml of  $\text{H}_2\text{SO}_4$  diluted to 100 ml with  $\text{H}_2\text{O}$ ), cool to ca  $15^\circ$ , and shake vigorously until the pentabromacetone has crystallized (lace-like deposit on walls of flask). Place in refrigerator at ca  $15^\circ$  overnight. Avoid temp. of less than  $15^\circ$ , since at lower temp. there is a tendency for the pentabromacetone to freeze on sides of flask. Proceed as directed under **XXVI, 31**, beginning "carefully" decant supernatant liquid."

**67****ASH—OFFICIAL<sup>29</sup>**

Use 3–5 g of prepared sample, **61**, and proceed as directed under 5 or 7.

68

CHLORIDES IN ASH—OFFICIAL.—See 91.

69

PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen.)

Determine N as directed under II, 21, 22, or 23, using 2 g of prepared air-dried ground sample, 61. Multiply percentage of N by factor 5.7 to obtain percentage of protein.

70

FAT (ACID HYDROLYSIS METHOD)—OFFICIAL.—See 11.

71

CRUDE FIBER—OFFICIAL

(For bread and other baked products not containing fruit.)

Proceed as directed under XXVII, 27.

72

SUGARS—TENTATIVE—See XXVII, 28 and 29.

73

HYDROGEN ION CONCENTRATION—OFFICIAL, FIRST ACTION—See 14.

EXPERIMENTAL BAKING TEST<sup>30</sup>—TENTATIVE

74

EQUIPMENT

(1) *Mixer*.—Hobart-Swanson.

(2) *Fermentation bowls*.—Graniteware "oatmeal bowls." Top diameter 14.5 cm, bottom diameter 5 cm, and depth 6.5 cm.

(3) *Fermentation cabinet*.—Should have accurate temp. control ( $\pm 0.5^\circ$ ) and maintain relative humidity of at least 75%.<sup>30</sup>

(4) *Baking pans*.—Tall or low form, made of tin, with following inside dimensions:

LOW FORM TINS				TALL FORM TINS			
		cm	cm		cm	cm	cm
Length	top	11.5	bottom 9.5	top	10.5	bottom 9.3	
Width	top	7.0	bottom 5.5	top	6.0	bottom 5.3	
Depth		5.0		{ends	6.8		
				{sides	8.5		

NOTE: Investigation has shown that the low form tins give significantly higher volumes, lower variability between replicates, and more uniform crumb grain and texture than do the tall form tins; they also correspond more closely to commercial pans. The low form tins are especially recommended for research work. In reporting results of baking tests the type of pan used should be specified.

(5) *Baking oven*.—Should maintain temp. of  $230^\circ (\pm 5^\circ)$  and preferably be equipped with rotating shelf.

(6) *Thermometers*.—

(a) *Fermentation cabinet and dough testing*.—A.A.C.C. official thermometer graduated from  $15^\circ$  to  $40^\circ$  or equivalent Fahrenheit range.<sup>31</sup>

(b) *Oven*.—A.A.C.C. official thermometer graduated from  $100^\circ$  to  $260^\circ$  or equivalent Fahrenheit range.

(7) *Volume measuring apparatus*.<sup>32</sup>—Should be accurately calibrated. A set of aluminum loaf models is convenient. It is suggested that four standards of ca 300, 400, 500, 600 ml be used. Plot these against loaf volume readings of measuring device to obtain calibration curve of apparatus.



## 75

PREPARATION OF YEAST SUSPENSION AND SALT-SUGAR SOLUTION<sup>43</sup>

As the absorption of flours exceeds 50% it is convenient to prepare stock soln of sugar and salt and yeast suspension of such strength that 25 ml of each contains the required quantities of these ingredients per loaf. The volume displacement of 3 g of fresh compressed yeast is 2.5 ml and that of 1 g of salt and 2.5 g of sugar when dissolved together to make a total volume of 25 ml is 1.86 ml. The quantities of yeast, salt and sugar, and H<sub>2</sub>O required to prepare solns for varying numbers of loaves are shown below:

76 *Table for preparing yeast suspension and salt-sugar solution required for specified numbers of loaves using 25 ml each per loaf*

NUMBER OF LOAVES	YEAST SUSPENSION		SALT-SUGAR SOLUTION		
	YEAST	WATER	SUGAR	SALT	WATER
	<i>grams</i>	<i>ml</i>	<i>grams</i>	<i>grams</i>	<i>ml</i>
5	15	112.5	12.5	5.0	115.7
10	30	225.0	25.0	10.0	231.4
15	45	337.5	37.5	15.0	347.1
20	60	450.0	50.0	20.0	462.8
25	75	562.5	62.5	25.0	578.5
30	90	675.0	75.0	30.0	694.2

NOTES: 1. Water added per loaf in form of stock solns:

(a) 25 ml of yeast suspension contains.....	<i>ml</i> 22.5
(b) 25 ml of salt-sugar soln contains.....	23.1

Allowance in computing absorption..... 45.6

2. Agitate yeast suspension before and during removal of aliquot.

3. Keep soln at temp. such that when mixed with flour and any extra H<sub>2</sub>O required, the doughs will come from mixer at 30° (±0.5°).

## 77

## BASIC FORMULA

*Flour*.—100.0 g (±0.1 g) on 15% moisture basis (85.0 g dry matter). Determine moisture by 130° air oven or vacuum oven method (2 and 4).

*Yeast*.—3.0 g (3%) fresh compressed yeast.

*Salt*.—1.0 g (1%) 99.5% pure.

*Sugar (sucrose)*.—2.5 g (2.5%).

*Water*.—Sufficient to yield dough of standard consistency (not too "tight" nor too "slack").

## 78

## STANDARD PROCEDURE

(1) *Mixing*:

(a) Place flour in bowl of mixer, add 25 ml each of yeast suspension and salt-sugar soln plus sufficient additional H<sub>2</sub>O to bring dough to desired consistency. If this is not accomplished before dough has formed, discard mix and repeat test. Mix 1 min. Doughs should come from mixer at temp. of 30° (±0.5°). If 100 g of flour yields too small a dough for thoro mixing, use a larger quantity and scale dough to proper weight after mixing.

(b) *Alternative procedures*:

If an official mixer is not available, use any method of mixing that will thoroly incorporate ingredients and produce smooth dough with minimum development of gluten. A Hobart mixer equipped with 2 dough arms or cake paddle may be sub-

stituted. Even hand mixing may be resorted to when a machine mixer is not available.<sup>34</sup>

(2) *Calculation of absorption.*—% absorption (15% moisture basis) =  $45.6 + W - (100 - F)$ , where  $W$  = ml of  $H_2O$  added and  $F$  = weight of flour.

(3) *Fermentation:*

	<i>minutes</i>
First punch after.....	105
Second punch after additional .....	50
Mold after additional.....	25
Total.....	180

Remove dough from mixing bowl, fold 20 times in hands, put in fermentation bowl, and place in fermentation cabinet. After 105 min. remove dough from bowl

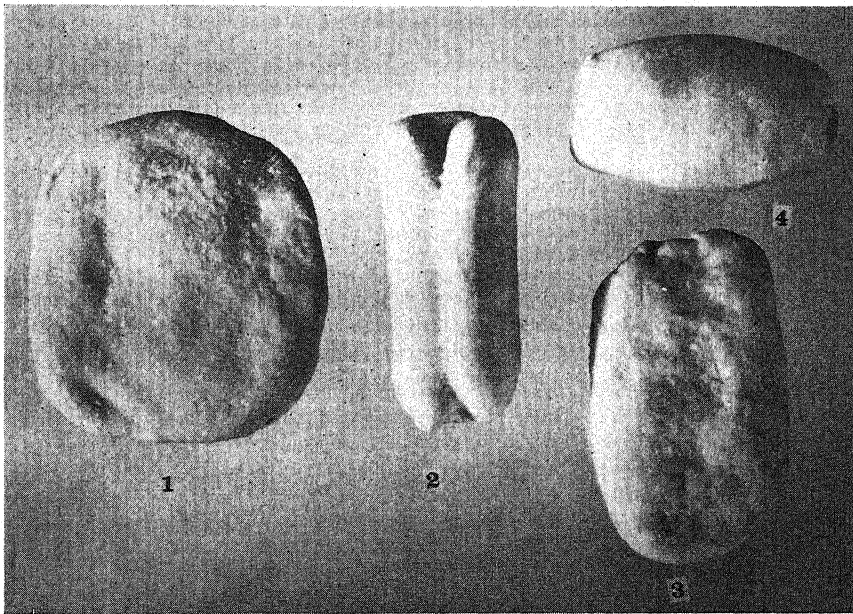


FIG. 24.—PREPARING THE DOUGH FOR PANNING

and fold 15 times in hands; round up and return dough to bowl and cabinet. Give second punch after additional 50 minutes' fermentation, folding dough 10 times and rounding up as before. After further fermentation of 25 min., mold dough and pan as directed.

(4) *Molding and panning.*—Place dough on piece of cotton or canvas belting. Press with heel of hand until dough is uniformly flat and circular (1). Loosen dough from belting and turn on reverse side. Fold over two opposite sides so that they overlap to considerable degree (2). Turn dough over and again flatten with heel of hand. Holding one end, again loosen from molding surface and turn on reverse side with seam of dough running from operator. Starting at more remote end, roll toward operator, folding as tightly as possible (3). Seal seam tightly and with seam on bot-

tom, seal ends by punching them vertically. Roll dough lightly under palm of hand, adjusting it to length of pan, and place in pan with the seam down. (Length of dough should not exceed that of pan prior to final light rolling.) *Use no dusting flour in molding process.* Grease pans very lightly and only when absolutely necessary to prevent loaves from sticking to sides of pans.

(5) *Proofing*.—Proof 55 min. under same conditions used for fermentation.

(6) *Baking*.—Bake 25 min. at temp. of  $250^{\circ} (\pm 5^{\circ})$  with thermometer placed at level of top of the baking pans at a distance of 5 cm on the side next the axis of rotation. (Precise control of temp. is essential.) Place open pan of  $H_2O$  in baking oven.

(7) *Measurement*.—Weigh loaf and measure its volume 30 min. after removal from oven. Place loaves in fairly air-tight cabinet until following morning.

(8) *Scoring*.—Score loaves the day following baking for external characteristics, crust color and symmetry, and for internal characteristics, crumb color, grain, and texture.<sup>35</sup>

NOTES: 1. The basic formula and standard baking procedure outlined are designed primarily for hard wheat flours to serve as point of reference and a basis for any supplementary tests that may be considered appropriate. Any additional testing procedure designed to reveal particular characteristics of flours may be used but only one variable at a time should be introduced. Such tests as mechanical modification by varying mixing time, addition of diastatic supplements, oxidizing agents as  $KBrO_3$ , and use of varying periods of fermentation have been found particularly valuable.

2. Fresh baker's yeast from same source should be used in each investigation, and supply should be stored in ice box in containers to prevent evaporation of moisture. The outside portions of yeast cake should be removed before being used. Studies on effect of aging yeast on loaf volume are somewhat contradictory, and it is advisable to secure a fresh supply every 2 days.<sup>36</sup>

3. It is advisable to prepare five "dummy" doughs at beginning and end of each regular day's baking in order to provide more uniform oven conditions at beginning and end of the series.

## 79

## SUPPLEMENTARY TESTS

(1) *Fermentation*.—Basic procedure varying fermentation time only.

(2) *Addition of  $KBrO_3$* .—Basic procedure with addition of  $KBrO_3$  in increments of 1 mg per loaf.

(3) *Sugar variation*.—Baking with increments of 2.5 g recommended for varying amounts of sugar in formula.

(4) *Mechanical modification*.—Variation of mixing time.

BAKED PRODUCTS OTHER THAN BREAD<sup>36</sup>—TENTATIVE

(Not containing fruit.)

80

SOLIDS.—See 62 and 63.

81

ASH.—See 5 or 7.

82

PROTEIN.—See 69.

83

FAT.—See 11.

84

CRUDE FIBER.—See XXVII, 27.

85

SUGARS.—See XXVII, 28 and 29.

86

HYDROGEN-ION CONCENTRATION—OFFICIAL, FIRST ACTION.—See 14.

## MACARONI PRODUCTS

87

COLLECTION AND PREPARATION OF SAMPLE<sup>37</sup>—OFFICIAL

Select from lot to be analyzed sufficient strips or pieces to assure a representative

sample, break these into small fragments with hands or in mill, and mix well. Grind 300–500 g in mill until all material just passes thru 20-mesh sieve. Keep ground sample in sealed container to prevent moisture changes.

#### TOTAL SOLIDS AND MOISTURE

88 *Vacuum Oven Method*<sup>38</sup>—Official

Using prepared sample, 87, proceed as directed under 3.

89 *Air Oven Method*—Official

Using prepared sample, 87, proceed as directed under 4.

90 ASH—OFFICIAL

Using 3–5 g of prepared sample, 87, proceed as directed under 5.

91 CHLORIDES IN ASH AS SODIUM CHLORIDE—OFFICIAL

Dissolve ash obtained under 90 in  $\text{HNO}_3$  (1+9), filter, wash filter paper with hot  $\text{H}_2\text{O}$ , and determine Cl in combined filtrate and washings as directed under XII, 35 or 37. Calculate Cl to its equivalent of NaCl. (This NaCl value deducted from total ash does not give NaCl-free ash.)

92 FAT (ACID HYDROLYSIS METHOD)<sup>39</sup>—OFFICIAL

Place 2 g of sample in Röhrig or Mojonnier fat extraction tube, add 2 ml of alcohol to prevent lumping on addition of acid, and shake so as to moisten all particles. Add 10 ml of HCl (25+11), mix well, set tube in water bath held at 70–80°, and shake at frequent intervals 30–40 min. Fill to within 1–2 ml of mark with alcohol and cool. Add 25 ml of ethyl ether and shake mixture well. Then add 25 ml of petroleum benzin (b.p. below 60°) and mix well. Let stand until upper liquid is practically clear and proceed as directed under 11, beginning “Draw off as much as possible.”

93 CRUDE FIBER—OFFICIAL.—See XXVII, 27.

94 PROTEIN<sup>40</sup>—OFFICIAL

Determine N as directed under II, 21, 22, or 23, using 1 g of prepared sample, 87. Multiply percentage of N by factor 5.7 to obtain percentage of protein.

95 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL—TENTATIVE.—See 32.

96 HYDROGEN-ION CONCENTRATION—OFFICIAL, FIRST ACTION.—See 14.

97 LIPOID AND LIPOID PHOSPHORIC ACID ( $\text{P}_2\text{O}_5$ )—OFFICIAL

Proceed as directed under 33 and 34.

#### UNSAPONIFIABLE RESIDUE—TENTATIVE

98 *Modified Kerr-Sorber Method*.—See 35.

99 EXTRACTION, SEPARATION, AND IDENTIFICATION OF COLORING MATTER IN MACARONI, EGG NOODLES, AND SIMILAR PRODUCTS<sup>41</sup>—OFFICIAL

Place ca 500 g of the coarsely ground sample (depending upon quantity of color present) in liter Erlenmeyer flask, add ca 700 ml of 80% alcohol, and shake at intervals 24 hours, or until color is imparted to solvent. Place flask with contents in refrigerator overnight to permit dissolved protein matter to precipitate out. Filter,

and evaporate filtrate to 100 ml. Add to filtrate ca  $\frac{1}{4}$  volume of 25% salt soln and slight excess of ammonia; cool, and transfer to separatory funnel. Extract this mixture with equal volume of petroleum benzin, b.p. below 60°, separate lower layer and repeat extractions with additional portions of solvent, until no more color is extracted. Reserve lower layer, if colored, for further treatment; if colorless, discard. Combine petroleum benzin extracts and wash with several small portions of ammonia water (1+50) to remove any material mechanically adhering to solvent. This ethereal soln will contain the fats, and also may contain the oil-soluble coal tar dyes, which may be identified by procedure under (1). If colored, immediately acidify alkaline aqueous soln, freed from fat and oil-soluble coal tar dyes, with acetic acid and extract in 25 ml portions with two 50 ml volumes of ether. The solvent, if colored, may contain turmeric, annatto, and trace of saffron. For their identification use procedure (2). If original aqueous soln freed from ether-soluble colors should still be colored, water-soluble dyes may be suspected, in which case the following procedure is recommended: Extract aqueous soln with 50 ml portions of amyl alcohol to remove balance of saffron, as well as common orange dyes (S & J numbers 85, 86, 13) and martius yellow. For their separation proceed as directed under 3. Draw off the lower aqueous layer, which, if colored, may contain naphthol yellow S, tartrazine, and sunset yellow. Extract these dyes with amyl alcohol after acidifying the soln with HCl to make ca 1 *N*. Remove tartrazine from solvent with 0.25 *N* HCl. Sunset yellow will also be removed at this stage with slightly lower acid concentration, and naphthol yellow S from nearly neutral soln. Confirm with wet and spot reactions. The extracted solns are usually very dilute, therefore it is advisable to concentrate by evaporation over steam bath, and if not clear, to add ca 5 ml of 25% salt soln to break up slight emulsions by precipitating protein matter. Filter and test filtrate by dyeing and coupling. This coupling test is carried out as follows: Treat ca 10 ml of filtered soln with excess of Br, destroy excess with saturated soln of hydrazine sulfate, and immediately pour into Na<sub>2</sub>CO<sub>3</sub> soln of alpha naphthol. In presence of tartrazine or sunset yellow a pink color will be produced. It is advisable to run a blank determination on above test for comparison.

(1) Extract original petroleum benzin extract with two or three 10 ml portions of mixture consisting of 1 part of HCl and 5 parts of acetic acid.

In presence of S & J numbers 7 or 16, yellow OB or yellow AB, a pink or red color is obtained. Test small portion of this acid extract with a few drops of SnCl<sub>2</sub>, which in the presence of the above dyes will cause either decolorization or decided fading. Dilute balance of acid extract with H<sub>2</sub>O, make slightly alkaline, and extract color with petroleum benzin. Wash solvent with 2-5 ml portions of H<sub>2</sub>O to remove excess alkali. Test ca 5 ml portion of the petroleum benzin extract with formaldehyde and acetic anhydride as directed under **XXI**, 9(a). Evaporate another 5 ml portion of the petroleum benzin extract to dryness in small evaporating dish and observe spot tests with HCl and H<sub>2</sub>SO<sub>4</sub>. Evaporate to dryness balance of petroleum benzin extract in small casserole and dissolve residue in dilute alcohol. Dye some silk strands, preferably using slightly alkaline soln. Compare spot tests obtained with Table 1, **XXI**. If they do not agree, a mixture of dyes may be present, which will necessitate separation according to the pH. The remaining coloring matters in the petroleum benzin extract may be due to the natural coloring matter of wheat, or to the coloring matter of egg. The coloring principle of egg yolk, lutein, when heated with alcoholic FeCl<sub>3</sub>, will produce a green coloration. However, this test is not specific for lutein, as carotene and xanthophyl give similar reactions.

(2) Wash the ether extract with 5 ml portions of H<sub>2</sub>O to remove excess of acid. To remove annatto and the traces of saffron, wash successively with 20 ml portions of 5% NaHCO<sub>3</sub> soln. Divide this alkaline soln into two portions. Heat one portion

to 60° on steam bath, dye the color on unmordanted cotton, and compare spot tests with a standard. Acidify remaining portion of the alkaline annatto soln with acetic acid and re-extract with ether. Divide ethereal extract into two small casseroles and evaporate to dryness. Dissolve contents of one casserole in 10 ml of ammonia water (1+9) and impregnate it on strip of cotton or filter paper. An orange yellow to an orange red coloration is obtained depending upon amount of dye present. Dry filter paper or cotton, add drop of 40%  $\text{SnCl}_2$ , and again dry. In presence of annatto a purple stain is produced. Spot contents of other casserole with  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ , when a blue and a greenish blue color are obtained. Transfer two portions (ca 10 ml each) of original ether extract from which annatto has been removed, into test tubes and treat with equal volume of 10%  $\text{NaOH}$  and equal volume of  $\text{HCl}$  (1+1), respectively. In presence of turmeric (curcuma) the alkaline soln will be reddish brown, while the acid soln will be red. Turmeric can further be confirmed by its behavior with boric acid. Apply this test as follows: Shake portion of original ether extract with equal volume of 70% alcohol and to this add 1/10 volume of  $\text{HCl}$ , mix, and divide soln equally into two test tubes. To one tube add a few crystals of boric acid and shake. Use other tube as a control. In the presence of turmeric, a red color will be produced after a short time.

(3) To separate and identify saffron and the orange coal tar dyes, dilute the amyl alcohol extract with two volumes of petroleum benzin and extract the mixed dyes with several 10 ml portions of  $\text{H}_2\text{O}$ . To a small portion of this aqueous extract add 1/10 volume of glacial acetic acid and add a few mg of dry sodium hyposulfite to reduce all the azo dyes. This treatment will not affect the saffron, which can then be re-extracted by amyl alcohol. After washing solvent repeatedly with small portions of  $\text{H}_2\text{O}$  (to remove decomposition products) evaporate to dryness, and confirm presence of saffron by spot tests. The remainder of color soln after addition of salt and acetic acid is re-extracted with amyl alcohol and later fractionated from the solvent for S & J numbers 85, 86, 13, by 5%  $\text{Na}_2\text{CO}_3$  soln. Martius yellow if present will still remain in the amyl alcohol and petroleum benzin after the removal of the saffron and oranges. In order to prove its presence, evaporate solvent to dryness and dissolve residue with 10 ml ammonia (1+9). Divide into two test tubes. Add carefully to one a few crystals of sodium hyposulfite. The presence of martius yellow will manifest itself by formation of pink soln. To check its presence use other subdivision for dyeing, spotting, etc.

#### SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 8, 424,664 (1925); 9, 39, 423 (1926).
- <sup>2</sup> Ibid., 8, 665 (1925); 9, 39 (1926).
- <sup>3</sup> Ibid., 8, 665 (1925); 9, 40 (1926).
- <sup>4</sup> Ibid., 7, 132 (1923).
- <sup>5</sup> Ibid., 19, 85 (1936); 20, 69 (1937); 22, 520 (1939).
- <sup>6</sup> Ibid., 19, 82 (1936); Cereal Lab. Methods, A.A.C.C., 1935.
- <sup>7</sup> J. Assoc. Official Agr. Chem., 6, 508 (1922); 9, 41 (1926).
- <sup>8</sup> Ibid., 10, 33 (1927); 11, 37 (1928); 17, 393 (1934); 18, 76 (1935); Determination of Hydrogen-ions, W. M. Clark, 3rd Ed., pp. 91, 94, 192-202.
- <sup>9</sup> J. Assoc. Official Agr. Chem., 22, 535 (1939); Cereal Chem., 14, 603 (1937).
- <sup>10</sup> U. S. Dept. Agr. Bur. Chem. Bull., 152, p. 104.
- <sup>11</sup> Ibid., 122, p. 54.
- <sup>12</sup> J. Assoc. Official Agr. Chem., 12, 39 (1929).
- <sup>13</sup> Ann. Phys. Chem., 85, 161 (1852).
- <sup>14</sup> J. Assoc. Official Agr. Chem., 7, 84 (1923); 12, 40 (1929); 14, 500 (1931).
- <sup>15</sup> Ibid., 7, 91 (1923); 9, 40 (1926).
- <sup>16</sup> Ibid., 7, 91 (1923); 9, 41 (1926).
- <sup>17</sup> Ibid., 9, 45 (1926); 10, 35 (1927); 11, 37 (1928).
- <sup>18</sup> Ibid., 8, 441 (1925); 10, 33 (1927).
- <sup>19</sup> Ibid., 10, 108 (1927); 11, 37 (1928); 16, 504 (1933); 17, 65 (1934).



- <sup>20</sup> Ibid., 22, 539 (1939).  
<sup>21</sup> Ibid., 18, 493 (1935); 19, 86 (1936).  
<sup>22</sup> Ibid., 17, 65 (1934).  
<sup>23</sup> Ibid., 15, 572 (1932); 16, 497 (1933); 17, 397 (1934); 18, 76 (1935); 19, 86 (1936); Cereal Chem., 9, 378 (1932); Am. Inst. Baking Bull. 8 (1932).  
<sup>24</sup> J. Assoc. Official Agr. Chem., 18, 76 (1935); 20, 69, 380 (1937); 22, 76 (1939); Cereal Chem., 11, 121, 299 (1934); Nebraska Agr. Expt. Sta. Bull. 8 (1916).  
<sup>25</sup> J. Assoc. Official Agr. Chem., 17, 329 (1934).  
<sup>26</sup> Ibid., 9, 42 (1926); 15, 72 (1932); 17, 65 (1934).  
<sup>27</sup> Ibid., 18, 574 (1935); 19, 86 (1936).  
<sup>28</sup> Ibid., 16, 427 (1933); 19, 86 (1936).  
<sup>29</sup> Ibid., 9, 42 (1926).  
<sup>30</sup> Ibid., 12, 41 (1929); Cereal Lab. Methods, A.A.C.C., 1935, p. 74; Cereal Chem., 7, 307, 341, 346 (1930); 8, 233 (1931).  
<sup>31</sup> Cereal Chem., 5, 158 (1928); 7, 362 (1930).  
<sup>32</sup> Ibid., 7, 307, 346 (1930).  
<sup>33</sup> Ibid., 5, 470 (1928).  
<sup>34</sup> Ibid., 158.  
<sup>35</sup> Ibid., 289; 6, 164, 253 (1929); 10, 545 (1933).  
<sup>36</sup> Ibid., 10, 617 (1933); Canadian J. Res., 6, 614 (1933).  
<sup>37</sup> J. Assoc. Official Agr. Chem., 9, 43, 396 (1926).  
<sup>38</sup> Ibid., 9, 43, 397 (1926).  
<sup>39</sup> Ibid., 6, 508 (1923); 11, 38 (1928).  
<sup>40</sup> Ibid., 9, 43 (1926).  
<sup>41</sup> Ibid., 6, 12 (1922); 8, 109 (1924); 15, 367 (1932); 19, 83 (1936); U. S. Dept. Agr. Bull. 448.

## XXI. COLORING MATTERS IN FOODS—TENTATIVE

(The numbers in parentheses and brackets following the name of a dye represent in the first instance the number of that dye as listed in "A Systematic Survey of the Organic Colouring Matters," founded on the German of Drs. G. Schultz and P. Julius, 1904, by Arthur G. Green, while the second number is that listed in the Society of Dyers and Colourists' "Colour Index," first edition, January, 1924.)

### 1

#### PIGMENTS AND LAKES

Separate the insoluble pigments, ultramarine, lampblack, etc., which are most commonly used as facings, by washing sample with  $H_2O$  and allowing washings to settle. Identify particles of coloring matter by microscopical examination and treat residue or purified coloring matter with chemical reagents.

The pigments occasionally encountered are charcoal or other form of carbon, ultramarine blue (principally aluminum, sulfur), prussian blue (principally iron), talcum (principally silica). Charcoal is indifferent towards the usual chemical reagents and can be burned. Ultramarine blue is stable towards alkalies, but is decomposed by dilute  $HCl$  with evolution of  $H_2S$ . Prussian blue is unaffected by dilute  $HCl$ , but is decomposed by alkalies. Talcum can be confirmed by the purple coloration obtained by fusing with cobalt nitrate (test for aluminum).

Lakes are products formed by combining organic coloring matters with metallic salts. They can be prepared from animal or vegetable coloring matters or from coal tar dyes. As a rule they are insoluble in  $H_2O$ , but are readily decomposed by acids with liberation of the coloring matter.

A large proportion of common pigments other than lakes, such as the yellow, brown and red ochres and umbers, are derivatives of heavy metals and contain Fe, Mn, etc. Others, such as the green and blue compounds, including certain green chlorophyll derivatives, may contain Cu. These pigments may be identified by usual tests for respective metals. The analytical properties of insoluble coloring matters are described in various standard works, some of which are listed under the selected references, especially Farbstofftabellen by Schultz<sup>1</sup> and Colour Index.

#### SOLUBLE COLORING MATTERS AND THEIR LAKES

##### SEPARATION BY WOOL DYEING PROCEDURE<sup>2</sup>

### 2

#### *Water-Soluble Coal Tar Dyes*

(a) *Wines, fruit juices, distilled liquors, flavoring extracts, vinegars, beers, sirups, non-alcoholic beverages, and similar products.*—Dilute 20–200 ml of sample with 1–3 volumes of  $H_2O$ , neutralize with  $NH_4OH$  (1+9) if necessary, and boil or heat on steam bath with small piece of white woolen cloth (nun's veiling). If mixture contains much alcohol, heat until most of it has been removed; in other cases take out wool after 5–15 min. and rinse with  $H_2O$ . Treat liquid with 3 or 4 drops of  $HCl$  for each 100 ml of soln and warm again 10–20 min. with a clean piece of wool. If the wool takes up much coloring matter in either case, the presence of coal tar dyes is indicated.

The basic colors dye the fiber best from neutral or faintly ammoniacal solns and, if present, they will appear on first piece of wool. Acid colors dye from neutral solns, but more readily from those containing free acid. The lichen colors<sup>3</sup> (archil, cudbear, litmus) go readily on wool, however, and many other natural colors, such as turmeric, will dye fiber if present in large amount. On the other hand, a few coal tar dyes, especially auramine O and naphthol green B, are quite unstable, and if present

in small quantities may give no distinct dyeing. Acid dyes are much more frequently used than basic dyes, and in most cases they may be removed from wool without much decomposition by "stripping" the latter with  $\text{NH}_4\text{OH}$  (1+9).<sup>4</sup> Many natural colors are destroyed by action of the alkali, while others remain unaffected on the fiber.

If behavior with wool in neutral and acid solns indicates presence of acid dyes, rinse colored cloth thoroly with  $\text{H}_2\text{O}$ , cover with  $\text{NH}_4\text{OH}$  (1+9) in a casserole, and boil for a few minutes. Remove cloth and squeeze out adhering liquid. Boil ammoniacal soln to remove excess of  $\text{NH}_3$ , drop in piece of clean wet wool, make distinctly but not strongly acid with  $\text{HCl}$  (1+9), and boil again. If acid coal tar dyes are present, they will usually give a fairly clean, bright dyeing on the second piece of wool. A further purification may be carried out by repeating the stripping and redyeing, tho this procedure is generally accompanied by a corresponding loss of dye.

(b) *Candies and similar colored sugar products.*—Dissolve ca 20 g of sample in 100 ml of  $\text{H}_2\text{O}$  and treat soln as directed under (a). When coloring matter is on surface of candy, pour off soln before colorless inner portion has dissolved.

(c) *Jams and jellies.*—Boil mixture of 10–20 g of sample and 100 ml of  $\text{H}_2\text{O}$  with wool in neutral and also in acid soln as directed under (a). For thick jams it is usually better, tho less easy, first to extract the coloring substances by treating product as directed under (d).

(d) *Canned and preserved fruits and vegetables, sausage casings, smoked fish, coffee, spices, etc.*—Macerate 20–200 g of sample with 4–5 times its weight of alcohol, 80% by volume. Allow to stand few hours, pour off solvent as completely as possible, and repeat extraction, using alcohol 70% by volume and containing ca 1% of  $\text{NH}_4\text{OH}$ . (1) Examine separately filtered alcoholic extracts as directed under (a); or, (2) boil ammoniacal soln until practically neutral, complete neutralization with acetic acid, add the neutral 80% alcohol extract, continue evaporation until most of the alcohol is removed, and boil a small portion with wool as directed under (a).

(e) *Cocoa and chocolate products.*—Treat cocoa as directed under (d). The alcoholic extract will contain large quantities of natural coloring matters, and several dyeings and strippings may be necessary to remove these in order to show presence of coal tar dyes.

Chocolate may be treated similarly, but the following procedure is preferable: Wash 20–200 g of well-divided sample with gasoline on filter until most of fat has been removed; if gasoline is colored, reserve for examination of oil-soluble dyes as directed under 3. Remove most of adherent solvent from residue by evaporation or pressure between layers of absorbent paper and digest with alcohol as directed under (d).

(f) *Cereal products (macaroni or other alimentary products).*—Use 500 g of coarsely ground sample and proceed as directed under XX, 99.

### 3

#### *Oil-Soluble Coal Tar Dyes<sup>5</sup>*

Prepare an alcoholic soln of the dye by applying one of following methods to oil or fat, obtained by extraction with ether or gasoline if nature of substance requires it:

(a) Shake oil or melted fat with equal volume of alcohol, 90% by volume, and wash alcoholic extract with several portions of gasoline to free coloring matter from foreign fats. The alcohol, after separation, will contain aniline yellow, butter yellow, aminoazotoluene, auramine, sudans, yellow OB, yellow AB, etc., if present.

(b) Saponify 20–200 g of the oil or fat with 0.5 *N* alcoholic  $\text{KOH}$ , remove most of the alcohol on the steam bath, and extract the soap with gasoline. Remove

dyes from solvent with 10 ml portions of mixture containing 1 part of HCl and 5 parts of glacial acetic acid. Most of common dyes are removed by this treatment, tho digestion with strong alkali may cause some decomposition and make extraction rather troublesome.

(c) Dilute 20–200 g of the oil or melted fat with 1–2 volumes of gasoline and shake out successively with 2–4% KOH or NaOH soln, HCl (1+3), and  $\text{H}_3\text{PO}_4$ - $\text{H}_2\text{SO}_4$  mixture, prepared by mixing 85%  $\text{H}_3\text{PO}_4$  with ca 10–20% by volume of  $\text{H}_2\text{SO}_4$ . The dilute alkali extracts sudan G (10) [23] and annatto (709) [1241]. The dilute HCl extracts aniline yellow (7) [15], aminoazotoluene (—) [17], and butter yellow (16) [19], the first two forming orange-red, the latter cherry-red solns in this solvent. The  $\text{H}_3\text{PO}_4$  mixture is necessary for the extraction of sudan I (11) [24], sudan II (49) [73], sudan III (143) [248], and the homologue of the last, sudan IV (—) [258], orange SS (—) [—], and oil red XO (—) [—]. Benzeneazo-beta-naphthylamine (—) [22] and homologues also come in this group, tho they readily undergo chemical changes in strongly acid mixtures. The procedure is not very suitable in presence of auramine, but this dye is seldom found in oils. Neutralize the fractions individually and re-extract with gasoline.

For the direct dyeing test use the alcoholic soln obtained as directed under (a). Evaporate to dryness the gasoline solns obtained as directed under (b) and (c) and dissolve residue in 10–20 ml of alcohol. To alcoholic soln add some strands of white silk and a little  $\text{H}_2\text{O}$  and evaporate on steam bath until alcohol has been removed or dye is taken up by the silk. The dyeing test is sometimes unsatisfactory, and in all cases small portions of the alcoholic soln should be tested by treating with equal volumes of HCl and  $\text{SnCl}_2$  soln. The common oil-soluble coal tar dyes are rendered more red or blue by the acid and are decolorized by the reducing agent. Most natural coloring matters become slightly paler with the acid and are little changed by the  $\text{SnCl}_2$  soln.

#### SEPARATION BY IMMISCIBLE SOLVENTS PROCEDURE<sup>6</sup>

##### 4

##### *Coal Tar Dyes in General*

The use of immiscible solvents for the separation of mixtures of coloring matters generally requires a systematic fractionation since many dyes do not differ very greatly in their solubilities in various solvents.

##### 5

##### PREPARATION OF SOLUTION

(a) *Water-soluble colors*.—Proceed as directed under 2, omitting fixation of the color on wool, and obtain an aqueous soln as free as practicable from suspended matter, alcohol, acids, alkalies, and salts. Liquids require no preparation except removal of any alcohol that may be present.

(b) *Water-insoluble lakes*.—If sample is in solid form, treat well-divided material with sufficient  $\text{H}_2\text{O}$  to form a paste.

(c) *Oil-soluble dyes*.—Proceed as directed under 3, preferably 3(a) or 3(c).

##### 6

##### *Basic Dyes*

Most basic dyes may be separated from mixtures by making alkaline with 10% NaOH soln and shaking with ether.<sup>7</sup> Use prepared soln, 5, for this purpose. Separate ether layer, which may or may not be colored; wash twice with a few ml of  $\text{H}_2\text{O}$  to remove excess of alkali; and shake with acetic acid (1+18), which will take up any dye present and form a colored soln. Altho this treatment may, to some extent, alter the common basic colors, it can be used for detection of methyl violet B (451) [680], magenta (448) [677], bismarck brown (197) [331], malachite green

(427) [657], and rhodamine B (504) [749]. With care auramine (425) [655] also may be separated in this way, tho it is quickly decomposed on standing in alkaline soln.

## 7

*Acid Dyes*

The following short procedure is often convenient for examination of mixtures of acid dyes: Make prepared sample, 5, strongly acid by adding  $\frac{1}{2}$  its volume of HCl and shake with amyl alcohol. Separate amyl alcohol soln and wash by shaking with successive portions of  $\frac{1}{2}$  its volume of H<sub>2</sub>O, reserving portions in separate test tubes or beakers. Because of varying acid content of the amyl alcohol these washings will show regular decrease in acidity, and the coloring matters will appear in maximum quantity in the different fractions according to their respective solubilities. Ponceau 6R (108) [186] is washed out chiefly while the acidity is still high, approximately normal. Amaranth (107) [184], brilliant scarlet (106) [185], tartrazine (94) [640], sunset yellow FCF, orange G (14) [27], and soluble blue (480) [707] appear when the washings have an acidity of ca 0.25 N, and palatine scarlet (53) [77], ponceau 2R (55) [79] and 3R (56) [80], ponceau SX, naphthol yellow S (4) [10], cochineal (706) [1239], crystal ponceau (64) [89], and azorubine A (103) [179] between  $\frac{1}{16}$  N and 1/256 N. When practically all acid is removed, orange I (85) [150], orange II (86) [151], and croceine orange (13) [26], begin to wash out, and less readily, orange IV (88) [143] and metanil yellow (95) [138]. Finally the unsulfonated coloring matters, such as erythrosine G (516) [772], erythrosine B (517) [773], and the rose bengals (520) [777] and (523) [779] are removed very slowly by H<sub>2</sub>O or not at all unless solvent is diluted with gasoline and dyes are removed with H<sub>2</sub>O containing a few drops of NH<sub>4</sub>OH. Acid yellow (8) [16] and brilliant yellow S (89) [144] are not very uniform in composition. They are partially taken up by amyl alcohol from acid soln and appear chiefly in the first washings. Indigotine (692) [1180] behaves somewhat similarly.

When it appears probable that only the coal tar dyes listed in the regulations for the enforcement of the Federal Food and Drugs Act<sup>8</sup> for use in food products are present, the following abridged procedure may be conveniently used for their separation:

**PERMITTED COAL TAR FOOD COLORS<sup>9</sup>**

(Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, tartrazine, yellow AB, yellow OB, orange SS and oil red XO.)

## 8

**PREPARATION OF SOLUTION**

(a) *For foodstuffs containing oil-soluble dyes.*—Proceed as directed under 3(a), evaporate the 90% alcoholic extract to dryness in casserole, treat residue with 40 ml of low-boiling gasoline, and shake gasoline soln with 2 or 3 portions of 5 ml each of 2–4% NaOH soln (to remove annatto, turmeric, etc., if present). The gasoline soln will contain the yellow OB, yellow AB, orange SS, and oil red XO.

(b) *For foodstuffs which contain no oil-soluble dyes or from which these dyes have been removed.*—Proceed as directed under 2, omitting fixation of color on wool, and obtain an aqueous soln as free as possible from suspended matter, alcohol, acids, alkalies, and salts. The dye soln should be preferably between 0.01 and 0.05%. The soln obtained in the examination of colored food products rarely requires further dilution, but with commercial food colors care must be taken that the concentration is not too great.

## SEPARATION

(a) *Yellow AB and yellow OB.*—Extract gasoline soln of these dyes, 8(a), 3 times with  $\frac{1}{2}$  its volume of 13 *N*  $\text{H}_2\text{SO}_4$ . Shake each acid extract successively with 2 portions (equal volumes) of low-boiling gasoline, using same 2 portions of gasoline for each acid portion. Extract each of 2 latter gasoline portions with 20 ml of 13 *N*  $\text{H}_2\text{SO}_4$ , using same acid portion successively for both gasoline portions. Finally extract second of these gasoline portions with another 20 ml portion of 13 *N*  $\text{H}_2\text{SO}_4$ . (Original gasoline soln has now been shaken with acid 3 times, the next gasoline portion 4 times, and the third 5 times.) Combine acid extracts, dilute with  $\text{H}_2\text{O}$ , re-extract with low-boiling gasoline, and evaporate solvent. Yellow AB will be found in a practically pure state. Combine gasoline solns (original and subsequent solns left after acid washings), wash with small portions of  $\text{H}_2\text{O}$  to remove excess of acid, and evaporate solvent. The yellow OB will remain as a residue. (This method is not absolutely quantitative, but it is sufficiently accurate to make a separation of either of the dyes with comparatively little contamination from the other.) The following color test may be applied to the separated dyes to confirm their identity: Shake 5 ml of neutral gasoline soln of dye in test tube with 5 ml of mixture of 1 part of 40%  $\text{HCHO}$  soln and 4 parts of acetic anhydride. Both coloring matters are extracted by the acetic anhydride, yellow AB giving in few seconds a red colored soln, and yellow OB, under the same conditions, giving orange colored soln.

(b) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine.*—To soln obtained under 8(b), add sufficient 25% salt soln to make concentration ca 10% and 1 part acetic acid to every 7 parts of soln. Extract with 3–50 ml portions of amyl alcohol. Draw off the lower layer and reserve for further treatment. Wash the amyl alcohol extract in rotation with 25 ml portions of 5% salt soln until washings are colorless or nearly so. Add washings to original aqueous soln. Dilute amyl alcohol extract with equal volume of gasoline and wash with 25 ml portions of  $\text{H}_2\text{O}$  until all color is extracted. The coloring matters obtained are orange I and guinea green B. For their separation see (1) below. Treat the amyl alcohol gasoline soln with 10 ml portions 0.1 *N*  $\text{NaOH}$  or with 10 ml portions of  $\text{NH}_4\text{OH}$  (1+9), which will remove erythrosine. Acidify the original soln and washings (from which the 3 named dyes were removed) with  $\text{HCl}$  (1 volume acid to 40 volumes of soln) and extract in 50 ml volumes with three 50 ml portions of amyl alcohol. Reserve lower aqueous layer for further treatment. Wash the amyl alcohol extract with 25 ml portions of 0.25 *N*  $\text{HCl}$  until washings are colorless or nearly so. Combine washings with aqueous soln above. Extract the amyl alcohol with several 25 ml portions of  $\text{H}_2\text{O}$  until all color is extracted. The coloring matters obtained are ponceau 3R, ponceau SX, and naphthol yellow S. For their separation see (2). Treat the original soln and washings (from which the 6 named dyes were removed) in 50 ml volume with 3–50 ml portions of  $\alpha$  dichlorhydrin. Reserve upper aqueous layer for further treatment. Wash the dichlorhydrin extract in rotation with several 20 ml portions of 25% salt soln. Combine washings with aqueous soln above. Dilute dichlorhydrin extract with 2 volumes of  $\text{CCl}_4$  and extract with several 25 ml portions of  $\text{H}_2\text{O}$  until all color is extracted. The coloring matters obtained are light green SF yellowish, fast green FCF, and brilliant blue FCF. For their separation see (3). Further acidify original soln and washings (from which the 9 named dyes were removed) with  $\text{HCl}$  (1 vol. acid to 40 vol. soln) and extract in 50 ml volumes with three 50 ml portions of amyl alcohol. (If color intensity of soln was not too strong, all coloring matter should have been extracted

by the solvent.) Discard lower colorless or nearly colorless layer and wash out dyes from the amyl alcohol extract in rotation with several 25 ml portions of  $H_2O$ , until all color is extracted. The coloring matters obtained are indigotine, amaranth, tartrazine, and sunset yellow FCF. For their separation see (4).

(1) *Orange I and guinea green B*.—Extract combined colors with two 20 ml portions of  $\alpha$  dichlorhydrin. Discard colorless upper aqueous layer, dilute solvent with 2 volumes of  $CCl_4$ , and extract out orange I in rotation with several 10 ml portions of  $H_2O$ , and guinea green B with several 10 ml portions of 25% alcohol.

(2) *Ponceau 3R, ponceau SX, and naphthol yellow S*.—Acidify combined colors with HCl (1 part acid to 10 parts of soln) and extract naphthol yellow S with two 20 ml portions of washed ethyl acetate or amyl acetate. (Ponceau 3R and ponceau SX are not extracted appreciably and remain in aqueous layer.) Wash solvent with 5 ml portions of normal HCl to remove traces of the ponceaus. Remove naphthol yellow S from the combined ethyl acetate or amyl acetate extracts with 5 ml portions of  $NH_4OH$  (1+9). Extract remaining ponceau soln with 20 ml portions of amyl alcohol and wash out excess of acid twice with a few ml portions of  $H_2O$ . Dilute amyl alcohol with an equal volume of gasoline, and remove color with small volumes of  $H_2O$ . Treat 10 ml of this soln with 1 ml of HCl, 2 ml of strong bromine  $H_2O$ , and lastly 3 ml of saturated hydrazine sulfate soln and immediately pour into test tube containing 10 ml of 2 N  $Na_2CO_3$  and 2 drops of 1% alcoholic alpha naphthol. (A light orange soln indicates ponceau 3R. A deep brownish red soln indicates ponceau SX.) Add to soln 5 ml of ether, mix well, and draw off the lower aqueous layer which, if colored, contains ponceau SX. To ethereal extract add equal volume of HCl when formation of purplish soln confirms presence of ponceau 3R.

(3) *Light green SF yellowish, fast green FCF, and brilliant blue FCF*.—Treat combined colors with equal volume of 2 N  $Na_2CO_3$  soln and extract in 25 ml volumes with two 50 ml portions of normal butyl alcohol. Draw off lower aqueous layer containing the fast green FCF and wash out last traces from solvent with 25 ml portions of 2 N  $Na_2CO_3$ . Reserve washings and add to aqueous soln for confirmatory tests. Light green SF yellowish is colorless in the solvent while brilliant blue FCF imparts a bluish green to it. To prove presence of light green SF yellowish in presence of brilliant blue FCF proceed as follows: Dilute solvent with equal volume of gasoline and remove color with small portions of  $H_2O$ . Treat 20 ml of soln with 4 ml of 10% NaOH and boil 5 min. Brilliant blue FCF is changed to a red phase, while light green SF yellowish is changed to a yellow. Acidify with 10 ml of glacial acetic acid, which changes brilliant blue FCF to a violet and light green SF yellowish to a green. Treat with ca 3 g of Zn dust and heat until soln is decolorized. Filter, make slightly alkaline with  $NH_4OH$  and later make acid with acetic acid and bring to boil. In presence of light green SF yellowish a deep green soln is formed while brilliant blue FCF remains colorless.

(4) *Indigotine, amaranth, tartrazine, and sunset yellow FCF*.—To separate indigotine heat a small portion of the soln, which should be neutral or faintly acid, to boiling, and add a few crystals of  $Na_2S_2O_4$  until all dyes are reduced. On adding a few drops of glacial acetic acid and shaking with air the indigotine is quickly restored, while amaranth, tartrazine, and sunset yellow FCF are destroyed. If a positive test for indigotine is obtained, add to remainder of mixed dye soln several decigrams of urea, heat, and while mixture is boiling add 1 or 2 drops of 10%  $NaNO_2$ . Indigotine is converted to the pale yellow isatine sulfonate, while amaranth, tartrazine, and sunset yellow FCF are but little affected. Acidify resultant mixture with  $H_2SO_4$  (1+4), using 1 part of dilute acid to 10 parts of soln. Extract in 25 ml portions with three 50 ml portions of normal butyl alcohol. Draw off lower layer and pass successively thru all funnels. Reserve aqueous layer if colored; if not colored,

discard. Prepare following soln: 13.5 ml of  $\text{H}_2\text{SO}_4$ , 100 g of anhydrous  $\text{Na}_2\text{SO}_4$ , and sufficient  $\text{H}_2\text{O}$  to make 1 liter. Extract the butyl alcohol successively with 25 ml portions of the soln until washings are colorless. Reserve them for amaranth and tartrazine. Dilute the butyl alcohol with equal volume of gasoline and remove sunset yellow FCF with  $\text{H}_2\text{O}$ . Confirm with dyeing tests and wet reactions.

Acidify reserved soln with  $\text{HCl}$  (1 vol. acid to 20 of soln) and extract with two 30 ml portions of amyl alcohol. (This will extract both amaranth and tartrazine while the isatine compound, being less readily extracted, remains in lower layer and is discarded.) Remove coloring matter with several 10 ml portions of  $\text{H}_2\text{O}$ . To a portion of the soln add 5 drops of  $\text{NH}_4\text{OH}$  and a few crystals of  $\text{Na}_2\text{S}_2\text{O}_4$ . (This treatment will destroy amaranth completely, leaving tartrazine practically unaltered.) Add excess of  $\text{HCl}$  and speedily extract dye with small volume of amyl alcohol, from which soln tartrazine can be removed with 0.25  $N$   $\text{HCl}$ . Treat another 10 ml portion of the neutral dye soln in test tube with 2 ml of 20%  $\text{NH}_4\text{Cl}$  and 1 ml of 25%  $\text{KCN}$  soln and heat in boiling water bath 5 min. Cool rapidly, acidify with 2 ml of  $\text{HCl}$ , and extract with 10 ml of amyl alcohol (caution). Draw off lower layer and discard. Remove tartrazine with 5 ml portions of 0.25  $N$   $\text{HCl}$ ; amaranth is converted to a lower sulfonated dye, and is not removed at that acid concentration. Dilute solvent with equal volume of gasoline and extract dye with small volumes of  $\text{H}_2\text{O}$  (amaranth is modified to brownish red dye).

## 10

IDENTIFICATION<sup>10</sup>

The most widely used tests for identification of coal tar dyes refer to changes produced with acids and alkalies. Other tests, based upon behavior with reducing agents, followed perhaps by treatment with oxidants or by separation and identification of the reduction products,<sup>11</sup> and tests based upon oxidation of the dye and treatment of the oxidation products,<sup>12</sup> are generally applicable. Spectroscopic methods are also used.<sup>13</sup>

## 11

*I. By Color Changes Produced with Acids and Alkalies*

Transfer separated coloring matter to wool (or to silk in case of oil-soluble dyes) by boiling as directed under 2(a) or 3. (Care should be taken that the final dyeing is made in a soln fairly free from foreign matter such as sugar or aromatic substances, which, adhering to the fiber, may modify the reaction. In most cases the quantity of color available is small and should not be used to dye too large a piece of wool, or silk.) Rinse the dyed fiber thoroly in running  $\text{H}_2\text{O}$ , dry, cut into small pieces, and place separately in depressions of white porcelain spot plate. Moisten pieces with  $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ , 10% soln of  $\text{NaOH}$ , and  $\text{NH}_4\text{OH}$  containing 12% by weight of  $\text{NH}_3$ . (For many coloring matters the hue upon treatment with acids or alkalies varies markedly with concentration of reagents and quantity of dye present; therefore the unknown dye should be compared with dyeings of known colors of approximately the same dye concentration as shown by their appearance.)

Table 1 shows color changes produced on wool dyed with 0.1–0.5% solns of the respective coloring matters. Included also are the reactions of the oil-soluble colors, but these refer to dyeing on silk. The dyes are arranged approximately according to hue. Brown is classed with orange; black (gray), with violet.

## 12

*II. By Special Tests*

(a) *Oil-soluble dyes (yellow AB, yellow OB, orange SS, and oil red XO).*—The alcoholic solns of these dyes become red on treatment with  $\text{HCl}$ ; are unaffected by alkalies; are reduced by  $\text{SnCl}_2$ ,  $\text{TiCl}_3$ , and  $\text{Na}_2\text{S}_2\text{O}_4$ ; and the color is not restored to reduced solns on addition of  $\text{FeCl}_3$  or  $\text{K}$  persulfate.



TABLE 1.—*Color reactions produced on dyed fibers by various reagents*

COLORING MATTER	C. I. NO.	S. & J. NO.	STRONG HYDROCHLORIC ACID	CONCENTRATED SULFURIC ACID	10% SODIUM HYDROXIDE SOLUTION	DILUTE AMMONIUM HYDROXIDE
Rhodamine B	749	504	Orange	Yellow	Bluer	Bluer
Rose Bengal	779	523	Almost decolorized	Orange	No change	No change
Archil	1242	710	Red	Reddish brown	Violet	Violet
Magenta	677	448	Yellowish brown	Yellowish brown	Decolorized	Paler
Acid Magenta	692	462	Almost decolorized	Yellow	Decolorized	Decolorized
Palatine Red	85	62	Darker	Blue	Dull brown	Little change
Bordeaux B	88	65	Violet	Blue	Brick red	Little change
Amaranth	184	107	Slightly darker	Violet to brownish	Dull brownish to orange red	Little change
Azorubine A	179	103	Little change	Violet	Red	Red
Erythrosine	773	517	Orange-yellow	Orange-yellow	No change	No change
Ponceau 6RB	286	169	Blue	Blue	Dull violet-red	Little change
Ponceau 6R	186	108	Violet-red	Violet	Brown	Orange-red
Crystal Ponceau	89	64	Red	Violet	Dull brown	Little change
Ponceau 3R	80	56	Little change	Little change	Dull orange	Little change
Ponceau SX	...	...	Deeper red	Deeper red	Orange yellow	Orange yellow
Sudan III*	248	143	Violet, then brown	Green	Violet-red	Little change
Safranin	841	584	Greenish blue	Green	Red	Red
Brilliant Scarlet	185	106	Red	Violet-red	Yellowish brown	Orange-red
Ponceau 2R	79	55	Little change	Little change	Brownish yellow	No change
Palatine Scarlet	77	53	Darker	Violet-red	Brownish yellow	No change
Erythrosine G	772	516	Yellow-orange	Yellow-orange	No change	No change
Sudan II*	73	49	Red	Violet-red	Little change	No change
Sudan I*	24	11	Orange-red	Red	Redder	No change
Cochineal	1239	706	Little change	Little change	Violet-red	Violet-red
Bismarck Brown	331	197	Redder, darker	Browner	Yellower	Yellower
Bismarck Brown R	332	201	Redder, darker	Browner	Yellower	Yellower
Orange I	150	85	Violet	Violet	Red, dark	Red, dark
Orange II	151	86	Red	Red	Dull red	No change
Croceine Orange	26	13	Orange-red	Orange	Slightly darker	No change
Orange G	27	14	Little change	Orange	Dull, brownish red	No change
Orthotoluenazobeta- naphthylamine* (Yellow OB)	61	...	Red	Violet	Little change	No change

\* Oil-soluble.

	22	...	Red	Violet	Little change	No change
Benzeneazobeta-naphthylamine* (Yellow AB)	...	...	Cherry red	Cherry red	Slightly yellow	No change
Orange SS*	..	..	Cherry red	Brownish yellow	Slightly yellow	No change
Oil red XO*	23	10	Orange-yellow	Orange-yellow	Orange-yellow	No change
Sudan G*	19	16	Violet-red	Orange-yellow	No change	No change
Butter Yellow*	15	7	Violet-red	Orange-yellow	Little change	No change
Aniline Yellow*	17	...	Dull orange	Orange-yellow	Little change	No change
Aminoazorthotoluene*	766	510	Little change	Little change	Green fluorescent	Green fluorescent
Fluoresceine	138	95	Violet-red	Violet	No change	No change
Metanil Yellow	145	92	Violet-red	Violet-red	Dull brown	Little change
Azoflavine	16	8	Red	Orange	Little change	No change
Acid Yellow S	144	89	Violet-red	Violet-red	Little change	Little change
Brilliant Yellow S	640	94	Slightly darker	Slightly darker	Little change	Little change
Tartrazine	...	...	Slightly redder	Slightly redder	Browner	Little change
Sunset yellow FCF	...	4	Almost decolorized	Very pale, dull brown	No change	No change
Naphthol Yellow S	10	...	...	...	...	...
Auramine	655	425	Decolorized	Almost decolorized	Decolorized	Paler
Turmeric	1238	707	Red	Reddish brown	Orange	Orange
Quinoline Yellow	801	667	Slightly darker	Brownish yellow	Slightly paler	Little change
Naphthol Green B	5	398	Yellowish	Brownish yellow	No change	No change
Guinea Green B	666	433	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Light Green SF Yellowish	670	435	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Fast Green FCF	...	...	Orange	Green to brown	Blue	Blue
Brilliant Blue FCF	...	...	Yellow	Yellow	No change	No change
Night Green 2B	667	438	Pale orange-yellow	Yellowish brown	Decolorized	Paler
Malachite Green	657	427	Almost decolorized	Almost decolorized	Decolorized	Decolorized
Erioglaucine A	671	436	Yellow	Pale, dull yellow or brown	Slightly darker	Little change
Patent Blue A	712	442	Pale orange-yellow	Green to brown	Little change	Little change
Soluble Blue	707	480	Paler	Brown	Pale reddish	Almost decolorized
Indigotine	1180	692	Slightly darker	Darker	Greenish yellow	Greenish blue
Formyl Violet	698	468	Pale orange-yellow	Pale, dull orange	Decolorized	Decolorized
Methyl Violet	680	451	Yellowish	Yellowish	Decolorized	Almost decolorized
Nigrosine, soluble	865	602	Dull bluish	Dull greenish	Brownish red, paler	Pale reddish

(b) *Water-soluble dyes (amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine).*—Treatment of these dyes in acid soln with  $\text{SnCl}_2$ ,  $\text{TiCl}_3$ , Zn dust, or  $\text{Na}_2\text{S}_2\text{O}_4$  decolorizes indigotine, amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, and tartrazine. With indigotine the color returns on shaking with air, but more readily on warming or on addition of  $\text{FeCl}_3$  or K persulfate. Excess of reducing agents must be avoided. With last 6 named dyes the color is not restored. Dilute solns of light green SF yellowish, guinea green B, fast green FCF, brilliant blue FCF, naphthol yellow S, and erythrosine become yellow or colorless with acid so that the effects of acid-reducing agents are not so readily apparent. Neutral solns of naphthol yellow S are first changed to pink and later decolorized by  $\text{Na}_2\text{S}_2\text{O}_4$  and other reducing agents, the color not returning with air or oxidants. Erythrosine, light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B become paler with  $\text{Na}_2\text{S}_2\text{O}_4$ , the color being partially restored upon addition of K persulfate.

In hot solns containing an excess of Na tartrate, water-soluble dyes are readily decolorized by  $\text{TiCl}_3$ .<sup>14</sup> In the case of indigotine, if the reducing agent has been added carefully and an excess avoided, the blue color readily returns on shaking with air. With erythrosine, light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B the color is scarcely restored by air, but on cooling and adding K persulfate it returns imperfectly. The reduction products of the other dyes do not give colored solns again on oxidation, if a slight yellowish or brownish tint that may sometimes appear is disregarded.

(1) *Light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B* belong to the triphenyl-methane type of dyes. Solns of light green SF yellowish and guinea green B behave similarly with acids, alkalies, and reducing agents, producing a yellow to a greenish yellow with mineral acids, and an almost colorless soln with alkalies as well as with reducing agents. On the other hand, while the reactions of fast green FCF and brilliant blue FCF are similar with acids and reducing agents, they differ in respect to their behavior to alkalies. While light green SF yellowish is decolorized by the addition of  $\text{NH}_4\text{OH}$  or 10% NaOH, fast green FCF produces a deep blue soln by similar treatment, which is not altered even on boiling; brilliant blue, on the other hand, is not affected by  $\text{NH}_4\text{OH}$  or fixed alkalies in the cold, but is changed to a reddish purple soln upon boiling with 10% NaOH. The easy solubility of these 4 colors in  $\alpha$  dichlorhydrin differentiates them from all other permitted dyes. To separate guinea green B from light green SF yellowish, fast green FCF and brilliant blue FCF, proceed as follows:

*Light green SF yellowish and guinea green B.*—Prepare soln of 250 g of NaCl, 27 g of crystallized Na acetate, and 24 ml of acetic acid in  $\text{H}_2\text{O}$ , and dilute to 1 liter.

To separate and differentiate the 2 green coloring matters add to every 20 ml of dye soln 1 ml of HCl and extract with an equal volume of amyl alcohol. Draw off lower layer and remove light green SF yellowish by washing remaining amyl alcohol portion with equal volumes of the NaCl-sodium acetate soln until no more color is extracted. Dilute the amyl alcohol with an equal volume of gasoline and remove the guinea green B with  $\text{H}_2\text{O}$ .

*Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—To separate and differentiate proceed as directed under 9(3).

(2) *Indigotine* is extracted in small proportions from slightly acid solns by shaking with  $\alpha$  dichlorhydrin, from which it may be removed with small portions of 25% salt soln. Most of other common bluish dyes are triphenyl-methane derivatives and are relatively more soluble in the solvent than in the aqueous layer. Indigo is readily

destroyed by boiling with a very small amount of a fixed alkali soln, by which treatment it may be readily eliminated from other coloring matters.

(3) *Ponceau 3R* gives in neutral or faintly acid solns a bluish red, flocculent precipitate with  $\text{BaCl}_2$  or Ba acetate, practically all the dye being removed from soln. Some of the soln obtained in separation, 9(b), may be used in this test, the free HCl first being neutralized with Na acetate; or better, it may be evaporated to dryness on steam bath to remove the acid and the residue taken up with a little  $\text{H}_2\text{O}$ . A brick red precipitate will be formed on standing, when a neutral soln of the dye is treated with a 20% soln of neutral Pb acetate. The soln should contain 0.005% or more of the dye.

(4) *Naphthol yellow S*, in solns containing an excess of  $\text{NH}_4\text{OH}$  or  $\text{Na}_2\text{CO}_3$ , becomes intensely rose-red on addition of  $\text{Na}_2\text{S}_2\text{O}_4$ , the color gradually fading again as complete reduction takes place. A red coloration is also produced if an aqueous soln of the dye is treated with a few drops of 40%  $\text{SnCl}_2$  and an excess of 20% KOH is added.

(5) *Tartrazine* is characterized by its comparative inactivity towards acids and alkalies, the soln of the dye being hardly altered by these reagents. An alkaline soln of the dye is reduced with  $\text{Na}_2\text{S}_2\text{O}_4$  only with difficulty. A concentrated neutral or slightly acid soln of the dye, when reduced with  $\text{SnCl}_2$  soln or Zn dust and made slightly alkaline and filtered, will develop a purple coloration on standing.

(6) *Orange I* can readily be recognized by its behavior toward reagents. With a large excess of HCl it produces a purplish-red; with alkali in large excess it produces a bright red soln.

(7) *Erythrosine* differs from most of the common dyes in that it contains I. To test for I, acidify soln with  $\text{H}_2\text{SO}_4$ , shake with ether, separate ether soln of the color, and evaporate to dryness in Pt dish after addition of a few drops of  $\text{Na}_2\text{CO}_3$  soln or sufficient to form the deep red Na salt. Hold dish containing residue in the Bunsen flame until organic matter is destroyed, take up residue with  $\text{H}_2\text{O}$ , acidify with  $\text{H}_2\text{SO}_4$ , and test for I in one of the usual ways, such as with Cl water and  $\text{CS}_2$  or  $\text{CCl}_4$ , or with starch paste and an oxidizing agent. It is useless to test for I with very small quantities of dye, but in most cases sufficient coloring matter can be separated from the food product to give satisfactory results.

## 13

## NATURAL COLORING MATTERS

As a class the natural coloring matters show much less tendency to dye animal fiber than do the common synthetic colors. In many cases the crude products used contain a number of colored substances, and a complete separation is not practicable. As dilute solns of most of the natural coloring matters are sensitive to alkalies, and some are sensitive to acids, such reagents must be used with care. Relatively few good tests are known for the common natural colors. Some of their most useful analytical properties<sup>15</sup> are given in Table 2.

The properties of pure preparations of the various natural coloring matters are described, for the most part, by Rupe,<sup>16</sup> and by Perkin and Everest,<sup>17</sup> reference being made in these works to the original literature. Properties of the chlorophylls and carotinoids are given by Willstätter and Stoll,<sup>18</sup> those of the coloring matters of the cornflower, rose, pelargona flower, larkspur, cranberry, whortleberry, purple grape, sloe, cherry, plum, radish, and red beet by Willstätter and coworkers.<sup>19</sup>

## 14

## SEPARATION

(a) *By extraction with ether from neutral solns.*—From neutral solns ether extracts carotin, xanthophyl (the pigments found in leaves, fats and oils, egg yolk, carrots,

etc.), the coloring matter of tomatoes and paprika, and green chlorophyl. The coloring matter remains in the ether soln on shaking with normal NaOH soln or normal HCl, no apparent change taking place, altho chemically the substances may be altered more or less by this treatment.

(b) *By extraction with ether from acid solns.*—From slightly acid solns ether extracts very readily and completely the coloring matter of alkanet, annatto, turmeric, and the red dyewoods, sandalwood, camwood, and barwood. It extracts in large proportions the flavone coloring matters of fustic, Persian berries and quercitron (after hydrolysis), as well as the coloring matter of Brazilwood and the green derivatives formed from chlorophyl by alkaline treatment. It extracts in relatively small quantity the coloring matters of logwood, archil, saffron, and cochineal. The coloring matters of this group are readily removed from ether by shaking with alkaline solns, but in most cases they rapidly undergo chemical change.

(c) *By extraction with amyl alcohol from acid solns.*—From slightly acid solns amyl alcohol extracts the major part of the coloring matters of logwood, archil, saffron, and cochineal. Amyl alcohol extracts in relatively small proportions caramel and the anthocyanins constituting the red coloring matter of the most common fruits.

#### IDENTIFICATION

15

##### *I. By Color Changes Produced with Various Reagents*

Evaporate to dryness the ether solns obtained under 14(a) and 14(b), warm the residue with a little alcohol, and dilute with H<sub>2</sub>O. Dilute the amyl alcohol soln obtained under 14(c) with gasoline and extract with H<sub>2</sub>O. To portions of these somewhat purified solns of the coloring matter apply the reagents in the following manner:

*Hydrochloric acid.*—Add to soln first 1 or 2 drops of strong HCl, then excess equal to 3–4 times volume of soln.

*Sodium or potassium hydroxide.*—Make soln slightly alkaline by adding a drop of 10% NaOH or KOH soln.

*Sodium hyposulfite.*—Add a small crystal of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>.

*Ferric chloride.*—Add a small quantity of freshly prepared 0.5% FeCl<sub>3</sub> soln very carefully, a small drop at a time, as the colorations are not obtained in some cases when an excess is used.

*Alum.*—Add to test soln  $\frac{1}{2}$  its volume of 10% K- or NH<sub>4</sub>-alum soln.

*Uranium acetate.*—Add 5% U acetate soln dropwise.

*Sulfuric acid on dry color.*—Evaporate small quantity of soln or of the coloring matter in porcelain dish. Cool thoroly and treat dry residue with 1 or 2 drops of cold H<sub>2</sub>SO<sub>4</sub>. The colorations are in some cases extremely transitory, and they may be observed only the instant the acid wets the residue.

Table 2 shows the behavior of certain of the natural coloring matters when treated in the manner described above.

16

##### *II. By Special Tests*

(a) *Chlorophyl.*—The “brown phase reaction”<sup>20</sup> may be useful for the characterization of chlorophyl, when this has not been previously treated with alkalis. Treat the green ether or petroleum benzin soln of the coloring matter with a small quantity of 10% soln of KOH in methyl alcohol. The color becomes brown, quickly returning to green.

(b) *Annatto.*<sup>21</sup>—Pour on a moistened filter an alkaline soln of the color obtained by shaking out the oil or melted and filtered fat with warm 2% NaOH soln. If annatto is present, the filter paper will absorb the color, so that when washed with

TABLE 2.—*Reaction of certain natural coloring matters to common reagents*

COLORING MATTER	STRONG HYDROCHLORIC ACID	10 PER CENT SODIUM HYDROXIDE SOLUTION	SODIUM HYPOSULFITE	0.5 PER CENT FERRIC CHLORIDE SOLUTION	10 PER CENT ALUM SOLUTION	5 PER CENT URANIUM ACETATE SOLUTION	CONCENTRATED SULFURIC ACID ON DRY COLOR
Logwood	Deep red with excess of acid	Violet to violet-blue	Almost decolorized, color returning imperfectly by reoxidation	Dark shades of violet, brown or black (the first hue often evanescent)	Rose-red (change rather slow)	Violet, quickly fading	Red, changing to yellow
Red woods (Brazilwood, Sandalwood, Camwood and Barwood)	Deep red with excess of acid	Violet-red	.....	Dark shades of violet, brown or black (the first hue often evanescent)	Rose-red (change rather slow)	.....	.....
Anthocyanins of red fruit colors	.....	Change to green, dull blue or slate color, usually very quickly becoming browner by oxidation	Anthocyanidins derived by hydrolysis, almost completely decolorized	.....	.....	.....	.....
Alkanet	.....	Deep blue	.....	.....	.....	Yellowish green	Violet-blue
Archil	Little or no change	Blue	Decolorized, color returning when shaken with air. Reaction more easily seen in alkaline solution	.....	.....	.....	Violet-blue
Cochineal	Little or no change	Violet	No marked change	Slightly darker	.....	Green	.....
Annatto	Remains orange. Little change	.....	Little affected	No marked change. Perhaps somewhat browner	.....	.....	Blue
Turmeric (solution in ether or alcohol characterized by pure yellow color and light green fluorescence)	Orange-red or carmine-red on addition of several volumes of concentrated acid	Orange-brown	Little affected	No marked change. Perhaps somewhat browner	Little change	Somewhat browner	Red
Flavone colors of fustic, Persian berries, quercitron, etc.	Becomes intensely yellow with 2-4 volumes of concentrated acid	Bright yellow	Little affected	Olive-green or black colorations	More strongly yellow; fustic, developing a green fluorescence	Orange colorations	Yellow to orange
Saffron	Little or no change	Remains yellow	Little affected	No marked change. Perhaps somewhat browner	Little change	Not affected	Blue
Carotin and Xanthophyl	Little change. Perhaps slightly paler	Little or no change	Little affected	.....	.....	.....	Blue, reaction obtained with difficulty
Green Chlorophyl	More brownish	"Brown phase reaction," 16(a)	.....	.....	.....	.....	.....
Caramel	Little or no change	Little change or slightly deeper brown	Slightly paler	No change	.....	.....	.....

a gentle stream of  $\text{H}_2\text{O}$  it will remain dyed a straw color. Dry filter, add a drop of  $\text{SnCl}_2$  soln, and again dry carefully. If color turns purple, presence of annatto is confirmed.

(c) *Turmeric*.—Treat an aqueous or dilute alcoholic soln of the color with  $\text{HCl}$  until shade just begins to appear slightly orange. Divide mixture into two parts and add some  $\text{H}_3\text{BO}_3$  powder or crystals to one portion. A marked reddening will be quickly apparent, best seen by comparison with the portion to which the  $\text{H}_3\text{BO}_3$  has not been added. The test may also be made by dipping piece of filter paper in the alcoholic soln of the coloring matter, drying at  $100^\circ$ , then moistening with weak soln of  $\text{H}_3\text{BO}_3$  to which a few drops of  $\text{HCl}$  have been added. On drying again a cherry-red color will be developed.

(d) *Cochineal*.—When presence of cochineal is suspected, acidify mixture with  $\frac{1}{3}$  its volume of  $\text{HCl}$  and shake with amyl alcohol. Wash amyl alcohol soln of the coloring matter 2–4 times with equal volumes of  $\text{H}_2\text{O}$  to remove  $\text{HCl}$ , etc. Dilute the amyl alcohol with 1–2 volumes of gasoline and shake with a few small portions of  $\text{H}_2\text{O}$  to remove the color. Divide combined aqueous extracts into 2 portions. To the first add, dropwise, 5%  $\text{U}$  acetate soln, shaking thoroly after each addition. In presence of cochineal a characteristic emerald-green color is produced.<sup>22</sup> The green coloration with  $\text{U}$  salts is not developed in the presence of much free acid. Therefore, add a little  $\text{Na}$  acetate before making this test, or a correspondingly large quantity of  $\text{U}$  acetate must be added. To the second portion add 1 or 2 drops of  $\text{NH}_4\text{OH}$ ; in the presence of cochineal, a violet coloration results. This, however, is not so characteristic as the first test as many fruit colors give almost identical reactions. Cochineal is not decolorized by  $\text{Na}_2\text{S}_2\text{O}_4$  either in an acid, neutral or alkaline soln (differs from orchil).

As cochineal lakes often contain tin, further examination for this metal should always be made when water-insoluble cochineal compounds seem to be present.

(e) *Orchil*.—This coloring matter is either sulfonated or unsulfonated. Unsulfonated orchil is readily extracted by amyl alcohol from a weak acid soln, while the extraction of the sulfonated color is incomplete even from a strongly acidified soln. The behavior of the color towards acids and alkalies is similar to cochineal, *e.g.*,  $\text{HCl}$  produces a yellow shade and alkalies produce a bluish shade.  $\text{Na}_2\text{S}_2\text{O}_4$  reduces orchil, but the color is restored by air oxidation (differing from cochineal). The characteristic property of orchil is to dye, strip, and redye wool readily.

(f) *Caramel*.—A number of tests have been developed for this coloring matter, most of them being based upon the insolubility in ether,  $\text{CHCl}_3$ , or amyl alcohol. Probably the most sensitive test is the Woodman-Newhall<sup>23</sup> modification of Amthor's test with a slight deviation. To 10–20 ml of a neutral soln of the color in a small centrifuge tube add 2 ml of 5%  $\text{ZnCl}_2$  and 2 ml of 2%  $\text{KOH}$  soln, stir well, and centrifuge. Pour off liquid, and to magma add 25 ml of boiling  $\text{H}_2\text{O}$ . Mix, centrifuge, and pour off liquid. Repeat this operation until aqueous wash liquor is colorless. Dissolve precipitate with 15 ml of 10% acetic acid, concentrate, neutralize carefully, and filter. Divide into 2 portions. To one add 3–5 volumes of paraldehyde in 50 ml glass-stoppered cylinder, and just sufficient absolute alcohol to form a homogeneous soln (avoid excess). Caramel will be indicated by formation of a brownish precipitate on standing. To the other portion of the caramel soln add an equal volume of a freshly prepared reagent consisting of phenylhydrazin hydrochloride, 2 parts;  $\text{Na}$  acetate, 3 parts;  $\text{H}_2\text{O}$ , 20 parts. A dark brown precipitate is formed in presence of caramel.

#### COMMERCIAL COAL TAR FOOD COLORS<sup>24</sup>

The regulations for listing and certifying coal tar colors, promulgated under the Federal Food, Drug, and Cosmetic Act of 1938 (Fed. Reg., 4 [1935]; 3936 [1939]),

contain a new nomenclature for coal tar colors permitted for use in foods, drugs, and cosmetics. The food color names are:

<i>New Name</i>	<i>Former Name</i>
FD&C Blue No. 1	Brilliant Blue FCF
FD&C Blue No. 2	Indigotine
FD&C Green No. 1	Guinea Green B
FD&C Green No. 2	Light Green SF Yellowish
FD&C Green No. 3	Fast Green FCF
FD&C Orange No. 1	Orange 1
FD&C Orange No. 2	Orange SS
FD&C Red No. 1	Ponceau 3R
FD&C Red No. 2	Amaranth
FD&C Red No. 3	Erythrosine
FD&C Red No. 4	Ponceau SX
FD&C Red No. 32	Oil Red XO
FD&C Yellow No. 1	Naphthol Yellow S
FD&C Yellow No. 2	Naphthol Yellow S-Potassium salt
FD&C Yellow No. 3	Yellow AB
FD&C Yellow No. 4	Yellow OB
FD&C Yellow No. 5	Tartrazine
FD&C Yellow No. 6	Sunset Yellow FCF

No doubt the increased interest in coal tar colors created by the Act will effect a revision of analytical methods. Pending such revision the colors are referred to in this chapter by their old names.

## 17

## PREPARATION OF SAMPLE

Thoroughly mix and without interruption weigh out portions required. If weighing cannot be made directly into the dish in which determination is to be made, use weighing bottles for this purpose, placing in each a quantity approximating the weight called for, and weigh immediately.

## 18

## MOISTURE

(a) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange 1, naphthol yellow S and its K salt, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Weigh ca 2 g of sample in a weighed Al dish 2" in diameter or in weighing bottle of about the same diameter, dry in air oven at 135° for 6 hours or overnight, cool over H<sub>2</sub>SO<sub>4</sub> in a desiccator, and weigh. Heat again 1 hour, cool in desiccator, and weigh. Repeat heating and weighing at hour intervals until weight becomes constant. Report loss in weight as moisture.

(b) *For yellow AB, yellow OB, orange SS, and oil red XO.*—Proceed as directed under (a), heating the dye to 80° instead of to 135° (100° for orange SS and oil red XO).

## WATER-INSOLUBLE MATTER.

## 19

## APPARATUS

*Prepared Gooch crucible.*—Digest a good grade of retentive asbestos with HCl (1+3), wash free from acid, and elutriate to remove fine particles. Prepare a well packed asbestos mat of suitable thickness in the Gooch, wash with hot H<sub>2</sub>O, dry, ignite, rewash, dry at 135°, cool in desiccator, and weigh. Repeat washing, heating, and drying until constant weight is obtained.



## 20

## DETERMINATION

(a) *Amaranth, ponceau SX, erythrosine, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Dissolve 5 g of dye in 200 ml of hot  $H_2O$  and allow soln to cool to room temp. Filter thru prepared Gooch crucible, wash with cold  $H_2O$  until all dissolved dye has been removed, dry at  $135^\circ$ , cool in desiccator, and weigh. Report the increase in weight as total insoluble matter.

(b) *Ponceau 3R, orange I, and indigotine.*—Dissolve 5 g of dye in hot  $H_2O$ , using 250 ml for ponceau 3R and orange I, and 500 ml for indigotine. Cool soln to room temp., let stand overnight, and filter with moderate suction. Wash with cold  $H_2O$ , dry, cool, and weigh as directed under (a).

## 21

## NON-VOLATILE WATER-INSOLUBLE MATTER

*Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Incinerate Gooch containing the total insoluble matter, 20, at low red heat until all organic matter has been volatilized. Cool in a desiccator and weigh.

## SODIUM CHLORIDE

## 22

## REAGENTS

All reagents must be halogen free.

*Sulfur dioxide soln.*—Saturate ice-cold  $H_2O$  with  $SO_2$ . Keep soln stoppered and in cold place.

## 23

## DETERMINATION

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Thoroughly mix 5 g of dye with 4–6 g of  $K_2CO_3$  or  $Na_2CO_3$  in 50 ml Pt, Ni, or porcelain crucible and moisten with  $H_2O$  or 50% alcohol. Cover evenly with ca 1 g of powdered carbonate, dry, and ignite at low red heat until organic matter is destroyed. Allow to cool and add enough  $H_2O$  to form thin paste. Break up any lumps present with glass rod to assure uniform suspension. Wash mixture into 250 ml volumetric flask with 100–150 ml of hot  $H_2O$  and allow to stand until all soluble salts are dissolved and mixture is cold. Dilute to mark with  $H_2O$ , mix thoroughly, and filter thru dry paper.

Place a 200 ml portion of filtrate in 600 ml beaker and add enough 6–7% soln of  $KMnO_4$  to oxidize sulfides and produce permanent pink color. Add ca 50 ml of  $H_2O$  and a slight excess of 10%  $AgNO_3$  soln (6–8 ml usually sufficient). Partially cover beaker with watch-glass and acidify soln by carefully adding ca 12 ml of  $HNO_3$ . Heat nearly to boiling, then add the saturated  $SO_2$  soln. Boil until any excess of  $SO_2$  is removed, cool, filter thru weighed Gooch crucible, wash precipitate of  $AgCl$  with  $HNO_3$  (1+99) and then twice with  $H_2O$ , dry crucible and its contents at  $135^\circ$ , cool in desiccator, and weigh. Calculate to percentage of  $NaCl$ .

(b) *Erythrosine.*—In 500 ml volumetric flask dissolve 5 g of the dye in 400 ml of  $H_2O$ . Precipitate color acid by adding mixture of 2 ml of  $HNO_3$  and 10–20 ml of  $H_2O$ , dilute to 500 ml, mix, and filter thru dry paper. Treat 200 ml of filtrate with slightly more 10%  $AgNO_3$  soln than is required to precipitate halogens present, add 5 ml of  $HNO_3$ , and heat to boiling. Cool, collect precipitate in weighed Gooch crucible, wash, dry, and weigh as directed under (a). If  $NaI$  is present, determine

as directed under 51, and subtract weight of AgI from weight of precipitate. Calculate the percentage of NaCl from net AgCl.

(c) *Naphthol yellow S*.—Dissolve 5 g of dye in ca 400 ml of H<sub>2</sub>O in 500 ml volumetric flask. Precipitate most of color with KOH (ca 30% soln), make to volume, mix, and filter. Neutralize 200 ml of the filtrate, make slightly acid with HNO<sub>3</sub>, and precipitate the chloride by adding a slight excess of 10% AgNO<sub>3</sub> soln. Boil for few minutes, cool, and filter thru weighed Gooch crucible. Wash, dry, and weigh precipitate and calculate as directed under (a).

(d) *Naphthol yellow S, K salt*.—Dissolve 5 g of dye in ca 800 ml of H<sub>2</sub>O in 1000 ml volumetric flask by heating on steam bath. Cool, and precipitate most of color with KOH (ca 30% soln). Dilute to mark and filter. To 400 ml of filtrate add HNO<sub>3</sub> until slightly acid, heat to boiling, and precipitate the chlorides with 10% AgNO<sub>3</sub>. Boil for few minutes, cool, and filter thru weighed Gooch crucible. Wash, dry, and weigh precipitate and calculate as directed under (a).

## 24

## SODIUM SULFATE

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, and indigotine*.—Transfer to 250 ml volumetric flask a volume of water soln that contains 5 g of the dye; add H<sub>2</sub>O, if necessary, to bring to 200 ml; and heat on steam bath. Add pulverized C. P. NaCl as follows: For amaranth, tartrazine, and sunset yellow FCF, 70 g; for ponceau 3R, ponceau SX, orange I, indigotine, 50 g. Stopper flask and shake at frequent intervals for an hour. (To hasten precipitation soln may be cooled in ice H<sub>2</sub>O.) Dilute to mark with saturated C. P. NaCl soln, shake, and filter on dry 18 cm paper. To 100 ml of filtrate add 200 ml of H<sub>2</sub>O and 1 ml of HCl (1+9), heat to boiling, and add slight excess of hot 2% BaCl<sub>2</sub> soln. Allow to stand overnight, filter thru weighed Gooch crucible, wash precipitate of BaSO<sub>4</sub> thoroly with hot H<sub>2</sub>O, dry, ignite, cool in desiccator, and weigh. Calculate weight of Na<sub>2</sub>SO<sub>4</sub> equivalent to BaSO<sub>4</sub> obtained.

(b) *Erythrosine*.—Use a 200 ml aliquot free of the color acid, 23(b). Precipitate and determine the BaSO<sub>4</sub> as directed under (a).

(c) *Light green SF yellowish, Guinea green B, fast green FCF, and brilliant blue FCF*.—Transfer to 250 ml flask a volume of soln that contains 5 g of dye; add H<sub>2</sub>O, if necessary, to bring to ca 200 ml, and heat on steam bath. Add 5 g of phosphotungstic acid and shake at intervals until dissolved. Then add 50 g of pure pulverized NaCl, shaking at intervals to dissolve the salt. Cool, dilute to mark with saturated pure NaCl soln, shake, and filter. To 100 ml of filtrate add 200 ml of H<sub>2</sub>O and 1 ml of HCl (1+9) and determine BaSO<sub>4</sub> as directed under (a).

(d) *Naphthol yellow S*.—Use 200 ml of filtered soln, 23(c), neutralize, and make acid with HCl. Precipitate and determine BaSO<sub>4</sub> as directed under (a).

(e) *Naphthol yellow S, K salt*.—Use a 400 ml aliquot of filtered soln, 23(d), make acid with HCl, and determine BaSO<sub>4</sub> as directed under (a).

## 25

## SODIUM ACETATE

*Brilliant blue FCF*.—Weigh 10 g of dye into 200 ml Kjeldahl flask, add 25 ml of H<sub>2</sub>O, and connect flask in upright position to vertical straight-tube water-jacketed condenser. Insert separatory or dropping funnel thru stopper of flask, together with tube leading from flask to condenser. Add 15 ml of H<sub>3</sub>PO<sub>4</sub> (sp. gr. 1.7) and heat contents of flask to boiling. Collect acetic acid and condensed steam in 300 ml Erlenmeyer flask to which has been added standard soln of NaOH. Continue boiling until ca 250 ml has been distilled over, replacing distillate by H<sub>2</sub>O from dropping funnel so that volume in Kjeldahl flask remains ca 20 ml. (This distillation should require ca 2 hours.) Remove receiver and titrate excess alkali with standard acid,

using phenolphthalein as indicator. Run blank distillation and deduct resulting acidity found. From corrected acidity calculate quantity of Na acetate present in dye.

26

## SULFATED ASH

Weigh accurately in weighing bottle ca 5 g of dye and transfer to Pyrex Kjeldahl flask or tall beaker, washing out weighing bottle with a little  $\text{H}_2\text{O}$ . Destroy organic matter to convenient extent by digestion, using 15 ml of  $\text{H}_2\text{SO}_4$  and adding  $\text{HNO}_3$  as required. As bulk of  $\text{HNO}_3$  is driven off, lower flame to avoid reaction on glass. Transfer mixture to weighed Pt dish and heat over ring burner, using at first a low flame at safe distance below dish, increasing flame, and bringing it closer to dish by gradual steps. Thus continue destruction of organic matter and volatilization of acids. Continue heating until production of acid fumes decreases. If C remains, remove flame, let mass cool a little, and add  $\text{H}_2\text{SO}_4$  dropwise until mass is moistened. Repeat treatment until the C is burned off and ash is white or reddish. Heat carefully with blast lamp until fusion takes place with production of a clear liquid free from bubbles. Cool in desiccator and weigh. After deducting weight of  $\text{Na}_2\text{SO}_4$  equivalent to inorganic Na salts (chlorides, sulfates, carbonates, etc.) found in other determinations, calculate to percentage of metallic Na combined in the dye.

27

## HEAVY METALS

Moisten the sulfated ash obtained under 26 with few ml of HCl and evaporate to dryness on steam bath. Warm residue with 20 ml of HCl (1+19) until all soluble material has dissolved, transfer to 100 ml volumetric flask, dilute to 100 ml, mix, and filter thru dry paper. Reserve two 40 ml aliquots for determination of Al, Ca, Fe, and Mg. Pour 20 ml of filtrate into test tube and pass in a washed stream of  $\text{H}_2\text{S}$  for 30 min. No turbidity other than that due to precipitated S should appear. If a colored precipitate is formed, filter and test it for Cu and Sn.

28

LEAD<sup>25</sup>

(Applicable to all permitted dyes.)

Place 5 g of dye in tall-form 500 ml Pyrex beaker, cover with watch-glass, add 15 ml of  $\text{HNO}_3$ , and let boil (or heat gently) till rapid evolution of brown fumes has ceased. Add 15 ml of  $\text{H}_2\text{SO}_4$  and continue heating. Add small quantities (1–2 ml) of  $\text{HNO}_3$  at intervals until organic matter is destroyed and soln is colorless or at most pale yellow. Continue heating, with evolution of dense white fumes, until very small quantity (3–5 ml) of soln remains in beaker. (Or digest as directed under 31.) Cool soln, and add 15–20 ml of  $\text{H}_2\text{O}$ . Re-evaporate soln thus formed to white fumes, cool, take up in 100 ml of  $\text{H}_2\text{O}$ , add 100 ml of 95% alcohol, and let stand overnight. Filter out precipitate of  $\text{PbSO}_4$ , which may be present in such small quantity as to escape detection with naked eye, and wash thoroly with 50% alcohol (ca 100 ml). Two 9 cm C. S. & S. No. 590 filter papers, or suitable fritted glass crucible, are satisfactory for retaining the  $\text{PbSO}_4$ .

Place filter paper in small beaker, add 20 ml of 40%  $\text{NH}_4$  acetate, and heat to boiling, breaking up paper with glass rod. Filter thru C. S. & S. No. 590 9 cm paper, or thru fritted glass crucible, into 100 ml colorimeter tube and wash with 4%  $\text{NH}_4$  acetate soln until 50 ml mark is reached. When filter paper is used for retaining the  $\text{PbSO}_4$  and the Pb content appears to exceed 10 p.p.m. use 40 ml of 40%  $\text{NH}_4$  acetate for dissolving the  $\text{PbSO}_4$  instead of 20 ml, wash with 4%  $\text{NH}_4$  acetate, filter into 100 ml volumetric flask, make to volume, mix, and use an aliquot portion in the colorimeter tube. Prepare standards containing known quantities of Pb for

comparison. To these add the same quantity of  $\text{NH}_4$  acetate as was used with sample and dilute all tubes to definite volume with  $\text{H}_2\text{O}$ . To each tube add 2 or 3 drops of glacial acetic acid and 10 ml of freshly prepared  $\text{H}_2\text{S}$  water. Shake tubes to insure thoro mixing and estimate quantity of Pb by comparison with standards. Run blanks on all reagents used.

29

IRON, ALUMINUM, CALCIUM, AND MAGNESIUM<sup>26</sup>

(Applicable to all permitted dyes.)

To one of the two portions reserved under 27, add 5 g of  $\text{NH}_4\text{Cl}$  and neutralize with  $\text{NH}_4\text{OH}$  (1+1), boiling to drive off any excess. If precipitate is very slight, it may be disregarded; otherwise, filter thru quantitative paper, wash with  $\text{H}_2\text{O}$  containing trace of  $\text{NH}_4\text{OH}$  (reserving filtrate and washings), and ignite paper and precipitate in weighed crucible. Weigh mixture of  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$ . Place mixed oxides in 500 ml Erlenmeyer flask and dissolve in aqua regia, boiling to drive off Cl. Add  $\text{H}_2\text{O}$  to bring volume to ca 75 ml and add  $\text{NH}_4\text{OH}$  to incipient precipitation. Dissolve precipitate with as little  $\text{HCl}$  as possible, cool, and titrate the ferric iron present with 0.1 *N*  $\text{TiCl}_3$  soln, 35, using 5 g of  $\text{NH}_4\text{CNS}$  as indicator. Calculate the Fe as  $\text{Fe}_2\text{O}_3$ . To calculate quantity of  $\text{Al}_2\text{O}_3$ , deduct weight of  $\text{Fe}_2\text{O}_3$  from total weight of mixed oxides. From weight of oxide calculate percentage of metallic Al. Pass washed stream of  $\text{H}_2\text{S}$  into alkaline filtrate from the Fe- and Al-hydroxides. A white precipitate indicates presence of Zn.

To other reserved portion add 250 ml of  $\text{H}_2\text{O}$  to insure low concentration of Mg, if present. Heat to boiling and add 3.5 g of  $\text{NH}_4\text{Cl}$  and enough  $\text{NH}_4\text{OH}$  soln (1+99) to make soln barely alkaline. Filter off precipitated hydroxides of Fe and Al. Wash and discard precipitate. Heat combined filtrate and washings to boiling and add 1 g of  $\text{NH}_4$  oxalate. After cooling and letting stand for an hour, filter thru asbestos mat prepared on small Witt plate in glass funnel and wash with very little  $\text{H}_2\text{O}$ , reserving combined filtrate and washings. Place mat in beaker, add 100 ml of  $\text{H}_2\text{O}$  and 2 ml of  $\text{H}_2\text{SO}_4$ , heat gently until Ca oxalate dissolves, and titrate with 0.1 *N*  $\text{KMnO}_4$  soln. Calculate as metallic Ca.

Heat to boiling reserved filtrate and washings and add a *N* soln of  $\text{NaNH}_4\text{HPO}_4$  until there is no further precipitation. While stirring add ca  $\frac{1}{3}$  the volume of  $\text{NH}_4\text{OH}$  (1+9). Let stand 3 hours, filter thru ashless paper, and wash with  $\text{NH}_4\text{OH}$  (1+49). Ignite filter and precipitate in weighed crucible, cool in desiccator, and weigh the  $\text{Mg}_3\text{P}_2\text{O}_7$ . Calculate as metallic Mg.

## ARSENIC

30

REAGENTS AND APPARATUS.—See XXIX, 1 and 2.

31

## DETERMINATION

Place 10 g of dye in 800 ml Kjeldahl flask. Moisten with  $\text{H}_2\text{O}$ , and add 20 ml of  $\text{H}_2\text{SO}_4$  and 10 ml of  $\text{HNO}_3$ . As soon as first violent reaction subsides, heat until most of brown fumes are expelled. Repeat addition of  $\text{HNO}_3$  (3–5 ml at a time) and heating until color is fairly well broken up and most of organic matter is in soln. Then add *cautiously, and in small portions*, 10 ml of 60%  $\text{HClO}_4$ . When violence of reaction has subsided, continue to add small quantities of  $\text{HNO}_3$  and heat as before until colorless soln is obtained. (If soln fails to clear up in 10–20 min. after addition of  $\text{HClO}_4$ , 3–4 ml more of this acid may be added and the  $\text{HNO}_3$  treatment continued until soln is colorless.) Boil 10–15 min., cool, transfer to 50 ml volumetric flask and make to volume with  $\text{H}_2\text{O}$ .

NOTE: Perchloric acid must be handled with care. The *anhydrous* acid is a strong oxidizing agent and may cause violent explosions in presence of organic matter, particularly alcohols. The mixture of  $\text{HClO}_4$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{HNO}_3$ , however, is apparently safe to use and has an oxidizing action much superior to the usual  $\text{H}_2\text{SO}_4$ - $\text{HNO}_3$  mixture.

Transfer aliquot not exceeding 25 ml to generator bottle, and neutralize with 25%  $\text{NaOH}$ . Add 5 ml of  $\text{HCl}$ , 5 ml of the  $\text{KI}$ , and 4 drops of the  $\text{SnCl}_2$ . From the standard  $\text{As}$  soln prepare standards corresponding to 0.010, 0.020, and 0.030 mg of  $\text{As}_2\text{O}_3$ , containing the same quantity of acid,  $\text{KI}$ ,  $\text{SnCl}_2$ , and  $\text{Na}_2\text{SO}_4$  as sample. Mix, allow to stand 30 min. at not less than  $25^\circ$ , or for 5 min. at  $90^\circ$ . Dilute to 40 ml.

Prepare generator as directed and center a strip of  $\text{HgBr}_2$  paper carefully in the narrow tube. According to the activity of the  $\text{Zn}$ , add to each standard and sample 10–15 g of activated stick  $\text{Zn}$  or 2–5 g of granulated  $\text{Zn}$  and add same quantity to each generator. Equalize as far as possible the surface area of  $\text{Zn}$  exposed in standard and sample.

Immerse apparatus to within 1" of top of narrow tube in water bath (kept at constant temp. at  $20$ – $25^\circ$ ) and allow evolution to proceed 1.5 hours. Remove strip and average length of stains on both sides in mm. Plot a graph of standard strips on cross-sectioned paper, using lengths in mm as ordinates and mg of  $\text{As}_2\text{O}_3$  as abscissa. (The preparation of a standard graph averages the errors of individual standards. Reading the strip from such a graph is considered more convenient and accurate than comparing the strips themselves.) Locate length of unknown strip on standard graph and read off on abscissa quantity of  $\text{As}$  present. Report to first decimal as p.p.m. of  $\text{As}_2\text{O}_3$ . Take smaller or larger aliquots when stain is longer or shorter than highest or lowest standard, respectively. Make frequent blanks. With reagents of suitable quality, blanks should not show more than 0.001 mg of  $\text{As}_2\text{O}_3$ .

#### ETHER EXTRACTIVES

32

##### REAGENTS

*Washed ether*.—Wash 1 liter of ether with 3 successive 150 ml portions of  $\text{H}_2\text{O}$  immediately before using.

33

##### DETERMINATION

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine*.—Place in separatory funnel that volume of soln that contains 10 g of the dye and add  $\text{H}_2\text{O}$ , if necessary, to bring volume to 200 ml, and, in the case of indigotine, to 500 ml. Extract with 2 successive 100 ml portions of the washed ether, shaking 1 min. during each extraction. Remove ether by decantation into clean funnel and rinse first funnel with 5 ml of ether, decanting into second funnel. Reserve color soln. Wash combined extracts with 20 ml portions of  $\text{H}_2\text{O}$  until washings are colorless. Decant ether into beaker, rinse funnel with 5 ml of ether, and decant into same beaker. Place beaker in dust-free atmosphere, allow ether to evaporate to volume of 50 ml, and transfer to weighed flat-bottomed 100 ml dish, previously dried to constant weight over  $\text{H}_2\text{SO}_4$  in desiccator. Rinse beaker with 5 ml of ether and drain into same dish. Let remainder of ether evaporate and dry over  $\text{H}_2\text{SO}_4$  to constant weight. The result represents the neutral extract.

To reserved color soln, add 2 ml of 10%  $\text{NaOH}$  soln and extract and rinse with ether. Reserve color soln. Wash combined ether extracts and rinsings with 20 ml portions of 0.1 *N*  $\text{NaOH}$  soln (1+99) until washings are colorless. Evaporate ether, dry, and weigh. The result represents the alkaline extract.

To the color soln reserved from the alkaline extraction, add twice the volume of HCl (1+3) necessary to neutralize. Repeat previous procedure, but do not reserve color soln. Wash ether extract with 0.1 N HCl (1+99) until washings are colorless. The result represents the acid extract.

(b) *Erythrosine*.—Determine as directed under (a), omitting acid extraction. In case of neutral extraction, wash combined ether extracts with three 20 ml portions of  $H_2O$ .

(c) *Naphthol yellow S, K salt*.—Proceed as directed in (a) but dissolve 5 g of dye in 500 ml of  $H_2O$ .

## 34

## SULFUR

*Amaranth, ponceau 3R, ponceau SX, orange I, naphthol yellow S (and its K salt), sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine*.—Place ca 0.2 g of the sample in Parr calorimetric bomb and mix thoroly with ca 10 g of  $Na_2O_2$ . Add a few mg of S-free sugar if necessary to aid in igniting the mass. Close bomb and ignite. When cool, open bomb, place it in 600 ml beaker, and cover beaker with watch-glass. Dissolve residue by adding warm  $H_2O$  thru lip of beaker until bomb is covered. Acidify soln cautiously with HCl and filter if necessary. Determine  $BaSO_4$  as directed under 24(a), beginning "heat to boiling, and add slight excess of hot 2%  $BaCl_2$  soln." Deduct the S equivalent to the  $Na_2SO_4$  determined under 24.

## COLOR ACID AND DYE

## I. By Titration with Titanium Trichloride

## 35

## REAGENT

*Standard titanium trichloride soln*.—To 200 ml of the commercial 15% soln of  $TiCl_3$ , add 150 ml of HCl and dilute to 2 liters. Make soln approximately 0.1 N, place in container with H atmosphere provision,<sup>27</sup> and allow to stand 2 days for absorption of residual O.

## 36

## STANDARDIZATION OF SOLUTION

*Method I*.—Prepare liter of 0.1 N  $Fe_2(SO_4)_3$  by dissolving ingot iron, Bureau of Standards Sample 55, in 30 ml of  $H_2SO_4$ . Dilute to ca 400 ml, adding slowly, with stirring, a soln of pure  $KMnO_4$  (3.16 g dissolved in ca 200 ml  $H_2O$ ) until faint but perceptible reddish tint results. The last few ml should be added dropwise. Cool and dilute to 1 liter. Measure 20 ml of the 0.1 N  $Fe_2(SO_4)_3$  into 500 ml flask, pass in strong stream of  $CO_2$ , and add the  $TiCl_3$  rapidly until near end point. Add 5 g of pure  $NH_4CNS$  and resume addition of  $TiCl_3$  carefully until the red color just disappears.

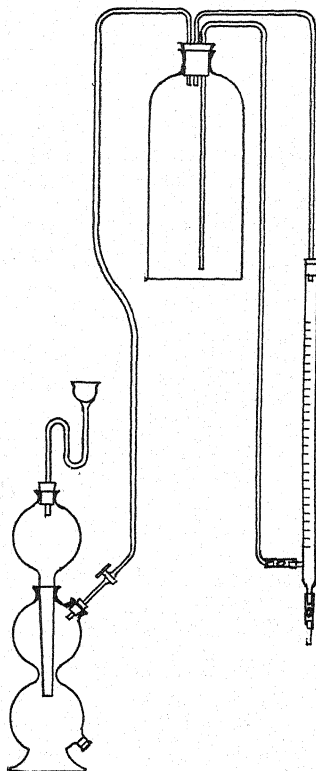


FIG. 25.—APPARATUS FOR TITRATION WITH TITANIUM TRICHLORIDE

*Method II.*—Make up 0.1 *N* soln of  $\text{KMnO}_4$  and standardize carefully, using Na oxalate, Bureau of Standards Sample 40, according to directions supplied with sample. Weigh 3 g of  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  and transfer to 500 ml flask. Introduce stream of  $\text{CO}_2$  and add 50 ml of recently boiled  $\text{H}_2\text{O}$  and 25 ml of 40% (by weight)  $\text{H}_2\text{SO}_4$ . Then, without interrupting current of  $\text{CO}_2$ , add rapidly 40 ml of the standardized  $\text{KMnO}_4$ . Add  $\text{TiCl}_3$  until near calculated end point. Then add quickly 5 g of  $\text{NH}_4\text{CNS}$ , and complete titration. Run blank on 3 g of  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , using the same quantities of  $\text{H}_2\text{O}$ , acid,  $\text{NH}_4\text{CNS}$ , and the current of  $\text{CO}_2$ .

## 37

## INDICATOR

For many dyes the  $\text{TiCl}_3$  titration end point is indicated by a sharp decolorization. For some dyes the change is so gradual that an excess of  $\text{TiCl}_3$  (not more than 0.3 ml of approximately 0.1 *N* soln) is required, and a suitable standard soln of some other dye must be used for the back titration, methylene blue serving well for this purpose. In other cases it is better to use an indicator which is reduced after original dye has reacted with the  $\text{TiCl}_3$ . Thus a known quantity of light green SF yellowish serves well for this purpose.

*Yellow AB and OB, orange SS, oil red XO, and indigotine.*—Prepare a 0.5% soln; for other dyes a 1% soln.

## 38

## DETERMINATION

(a) *Amaranth, ponceau 3R, and sunset yellow FCF.*—Place in 500 ml Erlenmeyer flask a volume of soln that corresponds to ca 20 ml of 0.1 *N*  $\text{TiCl}_3$ . Add 10 g of Na citrate and  $\text{H}_2\text{O}$  if necessary to bring volume to 150 ml. Heat to boiling, introduce stream of  $\text{CO}_2$  and titrate with standardized  $\text{TiCl}_3$ , keeping  $\text{CO}_2$  flow continuous to the end.

(b) *Orange I, ponceau SX, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Proceed as directed under (a), substituting 15 g of Na acid tartrate for Na citrate.

(c) *Naphthol yellow S and its K salt.*—Proceed as directed under (b), using as indicator a volume of light green SF yellowish standardized soln (freshly made) that contains 10 mg of dye. Run a blank on the tartrate, light green SF yellowish, and  $\text{H}_2\text{O}$ .

TABLE 3.—Quantities of color acids and of pure coal-tar dye equivalent to 1 ml of 0.1 *N* titanium trichloride solution

DYE	MOLECULAR WEIGHT OF COLOR ACID	COLOR ACID EQUIV- ALENT TO 1 ML 0.1 N $\text{TiCl}_3$	DYE EQUIVALENT TO 1 ML 0.1 N $\text{TiCl}_3$
Amaranth . . . . .	538.4	0.01346	0.01511
Ponceau 3R . . . . .	450.4	.01126	.01236
Ponceau SX . . . . .	436.3	.010907	.012006
Orange I . . . . .	328.3	.008207	.008756
Naphthol yellow S . . . . .	314.2	.002618	.002985
Naphthol yellow S-K salt . . . . .	314.2	.002618	.003253
Sunset yellow FCF . . . . .	408.3	.010206	.011305
Tartrazine . . . . .	468.3	.01171	.01336
Guinea green B . . . . .	668.5	.03342	.03453
Light green SF yellowish . . . . .	748.7	.03743	.03963
Fast green FCF . . . . .	764.5	.03822	.040423
Brilliant blue FCF . . . . .	748.5	.03743	.03963
Indigotine . . . . .	422.3	.02112	.02332

39

*II. By Precipitation*<sup>28</sup>

*Erythrosine*.—To a volume of H<sub>2</sub>O soln of sample that contains 0.25 g of dye, add, if necessary, sufficient H<sub>2</sub>O to bring volume to 100 ml. Add 5 ml of HNO<sub>3</sub> of approximately 0.6 N strength and filter thru weighed Gooch crucible. Wash thoroly with 0.5% HNO<sub>3</sub> and finally with not more than 10 ml of H<sub>2</sub>O. Do not allow precipitate to cake in crucible until washing has been completed. Dry to constant weight at 135°.

## PURE COAL TAR DYE

40 *I. By Direct Titration with Standard Titanium Trichloride Solution*

*Yellow AB, yellow OB, orange SS, and oil red XO*.—Dissolve 15 g of Na acid tartrate in 100 ml of H<sub>2</sub>O and add 0.1 g of dye dissolved in 100 ml of alcohol. Titrate with the standard TiCl<sub>3</sub> soln, 35, under CO<sub>2</sub>, using as indicator 10 mg of light green SF yellowish from fresh standardized soln, as directed under 38(c). Run blank as directed under 38(c), including also the 100 ml of alcohol. 1 ml of 0.1 N TiCl<sub>3</sub> = 0.006180 g of yellow AB, 0.006530 g of yellow OB, 0.006557 g of orange SS, and 0.00691 g of oil red XO. Calculate the percentage of pure dye.

41

*II. By Precipitation*

*Erythrosine*.—Multiply percentage of color acid obtained under 39 by factor 1.074.

42

## MATTER INSOLUBLE IN CARBON TETRACHLORIDE

*Yellow AB, yellow OB, orange SS, and oil red XO*.—In 100 ml beaker mix 5 g of the dye with 50 ml of CCl<sub>4</sub>, stir, and heat to boiling. Wash Gooch crucible prepared as directed under 19 with CCl<sub>4</sub> and heat at 100–105° to constant weight. Filter the hot dye soln thru crucible, transferring to it residue in beaker, and wash with five 10 ml portions of CCl<sub>4</sub>. Dry at 100–115° and weigh.

43

## WATER-SOLUBLE MATTER

*Yellow AB, yellow OB, orange SS, and oil red XO*.—Place 10 g of the well-powdered dye in 500 ml separatory funnel, add 100 ml of benzene, stopper, and mix until dissolved. Extract with two 100 ml portions of H<sub>2</sub>O, and evaporate 100 ml of extract in weighed Pt or crystallizing dish on steam bath. Dry in oven at 100–105°, cool, and weigh. The result represents the neutral extractive. Test small portions of remainder of filtrate for chlorides, sulfates, and nitrates. If more than traces are present, make proper analyses on aliquot portions of the filtrate.

## MELTING POINT

*Yellow AB, yellow OB, and orange SS*.

44

## APPARATUS

The apparatus, Fig. 26, consists of tube ca 15 cm long and 3.5 cm in internal diameter, with bulb of 5 cm internal diameter. Fill with glycerol to about height indicated. Fit tube with cork stopper carrying glass tube (A), 5 mm in diameter, which reaches nearly to bottom of bath; an ordinary test tube (B) in which a thermometer (C) is suspended by means of rubber stopper in such manner that Hg column is wholly within tube and Hg bulb equidistant from its walls; a long stemmed thermometer (D), supported so as to reach short distance below tube (B); and an outlet tube (E) to permit escape of air and vapor.



45

## DETERMINATION

To a capillary tube of 1 mm or smaller internal diameter, sealed at one end, transfer small portion of sample by inserting into sample open end of capillary, removing, inverting, and gently tapping until the well packed substance fills bottom of tube to height of 2–4 mm. Attach capillary tube to thermometer (C) by means of small rubber band, so that sample is placed at about middle of Hg bulb. Replace thermometer in tube, connect tube A to air blast, and force fairly rapid stream of air bubbles thru bath. Raise temp. of bath rapidly to within 5° of approximate melting point of sample. Keep temp. constant until thermometer reading is within 1° of that of bath. Then raise temp. slowly until melting point is observed. On approaching within 0.5° of melting point, the substance darkens; the true melting point is indicated by formation of meniscus on upper surface. When this condition is observed, hold temp. as nearly constant as possible until whole sample has liquefied.

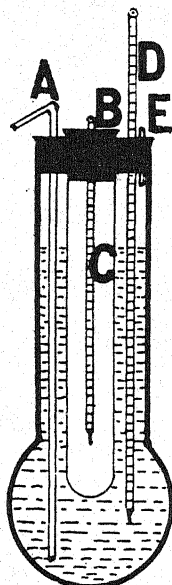


FIG. 26.—APPARATUS FOR DETERMINATION OF MELTING POINT

## LOWER SULFONATED DYES

46

## REAGENT

*Salt acetate soln.*—Dissolve 125 g of NaCl in H<sub>2</sub>O, add 12 ml of glacial acetic acid and a soln of 13.6 g of Na acetate, and dilute to 500 ml.

47

## PREPARATION OF SOLUTION

(a) *Amaranth, ponceau 3R, ponceau SX, sunset yellow FCF, tartrazine, and indigotine.*—Prepare H<sub>2</sub>O soln of such strength that 50 ml will contain 0.2 g of dye.

(b) *Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Prepare H<sub>2</sub>O soln of such strength that 10 ml will contain 0.1 g of dye.

48

## DETERMINATION

(a) *Amaranth and tartrazine.*—To 50 ml of prepared soln, 47(a), add 1 ml of HCl. Extract lower sulfonated dye by shaking soln successively in 3 separatory funnels, each containing 50 ml of amyl alcohol. Wash the amyl alcohol extracts by shaking successively with three 50 ml portions of 0.25 N HCl until the washings are practically colorless. Dilute amyl alcohol in each funnel with 100 ml portions of gasoline (sp. gr. 0.65), and remove lower sulfonated dye by washing with several 10 ml volumes of H<sub>2</sub>O, passing each portion thru the 3 funnels in an order the reverse of that previously followed.

Determine dye in H<sub>2</sub>O extract by titration against standard TiCl<sub>3</sub> soln, 35, using 10 g of Na citrate and volume of 100 ml. Run blank determination on all reagents, using 1 mg of the dye concerned. Calculate result to percentage of fast red E in amaranth and to fast yellow G in tartrazine. 1 ml of 0.1 N TiCl<sub>3</sub> soln = 0.01256 g of fast red E and 0.01081 g of fast yellow G. If quantity of dye is very low, it may be determined colorimetrically, amaranth or tartrazine, as appropriate, being used as standard.

(b) *Ponceau SX and sunset yellow FCF.*—Proceed as directed under (a), substituting 5% salt soln for 0.25 N HCl, and determine quantity of dye colorimetrically by comparison with standard soln of the dye.

(c) *Ponceau 3R*.—Proceed as directed under (a), substituting mixture of equal volumes of amyl alcohol and gasoline (sp. gr. 0.65) in place of the amyl alcohol and running the blank with 1 mg of ponceau 3R. Calculate result to percentage of Na trimethyl benzene-azo- $\beta$ -naphthol sulfonate, using factor 0.009807.

(d) *Indigotine*.—Proceed as directed under (a), substituting 0.25 ml of acid for 1 ml and Na acid tartrate for Na citrate, washing with 0.0625 *N* instead of 0.25 *N* acid, and running blank with 1 mg of indigotine. Calculate result to percentage of Na indigo monosulfonate, using factor 0.01821.

(e) *Light green SF yellowish*, *fast green FCF*, and *brilliant blue FCF*.—To 10 ml of prepared dye soln, 47(b), add 40 ml of the salt acetate soln and extract successively in 3 separatory funnels, each containing 100 ml of amyl alcohol. Wash extracts with 100 ml portions of the salt acetate soln, passing each wash portion successively thru the 3 funnels in the order used for original extractions. Remove dye from alcohol as directed under (a) and determine colorimetrically by comparison with a standard guinea green B soln of approximately same strength for light green SF yellowish, by comparison with a standard soln of fast green FCF and brilliant blue FCF for subsidiary dyes in the latter. Report as percentage of guinea green B in light green SF yellowish and subsidiary dye in fast green FCF or brilliant blue FCF.

## 49

BOILING RANGE OF  $\Psi$  CUMIDINE FROM PONCEAU 3R

(a) Dissolve 60 g of dye in 600–700 ml beaker with ca 450 ml of boiling  $H_2O$ , and add the hot soln very slowly to warm (60–80°) soln of 100 g of  $SnCl_2$  in 100 ml of HCl in tall liter beaker. Add dye soln in 10–20 ml portions, waiting after each addition until mixture is pale brown; otherwise dye will be precipitated, in which case it can be reduced only with difficulty. As reduction proceeds and soln becomes more dilute, heat to boiling, taking care that mixture does not boil over after each addition of dye, as some heat is generated by the reaction. After all the dye has been added and reduced, allow mixture to cool, and make alkaline by addition of ca 75 g of NaOH dissolved in 150–200 ml of  $H_2O$ .

Cool alkaline mixture and extract cumidine by shaking it with three 200 ml portions of ether. Combine ether extracts thus obtained and wash with  $H_2O$  until alkali and salts are removed. Evaporate solvent on steam bath, but avoid such prolonged heating as may tend to volatilize the base. Transfer residue of crude cumidine to small side-necked flask and distil it, carefully avoiding overheating. Observe range within which the substance volatilizes.

(b) Proceed as directed under (a) to directions for extraction with ether. Then steam distil the alkaline mixture until no more oil is carried over. Extract distillate with two 150 ml portions of ether. Wash combined extracts with successive 10 ml portions of  $H_2O$  until alkali and salts are removed. Evaporate solvent and complete the determination as directed under (a).

## 50

## ISOMERIC AND SIMILAR DYES IN AMARANTH

Take a volume of  $H_2O$  soln of sample that contains 0.1 g of dye and dilute, if necessary, to 40 ml with  $H_2O$ . Add 10 ml of 0.1 *N* benzidine soln (9.2 g of base per liter in 0.5 *N* HCl), mix well, and allow to stand exactly 2 min. Filter thru fluted paper and dilute 10 ml of filtrate to 100 ml. Compare this soln colorimetrically with standard amaranth soln containing 0.4 mg of the dye per 100 ml. The soln of the amaranth to be tested may be used in making the standard soln. If, after the benzidine treatment, the soln obtained is not more intensely colored than the standard soln, the proportion of isomeric dyes may be considered to be below 1.5%.

51

## SODIUM IODIDE

*Erythrosine*.—Dilute to ca 400 ml a volume of  $H_2O$  soln of sample that contains 5 g of dye and add mixture of 2 ml of  $HNO_3$  and 10–20 ml of  $H_2O$ . Dilute to exactly 500 ml, mix, and filter thru dry paper. Place 200 ml of filtrate in porcelain casserole and make slightly alkaline with 10%  $NaOH$  soln. Add ca 20 ml of 7%  $KMnO_4$  soln, mix, and add 10 ml of  $HNO_3$ . Place on steam bath and evaporate to dryness. Add 5 ml of 7%  $KMnO_4$  and 5 ml of  $HNO_3$  and again evaporate to dryness. Then add ca 50 ml of  $H_2O$ , 5 ml of  $HNO_3$ , and 25–30 ml of a saturated soln of  $SO_2$ . Stir frequently, breaking up any lumps, until hydrated oxide of Mn has dissolved. Filter, wash paper with  $H_2O$ , add to combined filtrate and washings an excess of 10%  $AgNO_3$  soln, and boil until  $SO_2$  has been expelled. Collect precipitate on weighed Gooch crucible, wash first with  $HNO_3$  (1+99), then twice with  $H_2O$ , dry, and weigh. Calculate as percentage of  $NaI$ .

52

## IODINE ORGANICALLY COMBINED

*Erythrosine*.—Place in porcelain casserole a volume of  $H_2O$  soln of sample that contains 0.3–0.4 g of dye. Add 5 ml of 10%  $NaOH$  soln and 35 ml of 7% soln of pure  $KMnO_4$ , and mix. Partially cover vessel with watch-glass and add 10 ml of  $HNO_3$ . Place on steam bath and keep covered until spattering ceases; remove watch-glass and allow evaporation to proceed to dryness, taking care to prevent access of reducing gases or vapors to mixture. Treat residue with 5 ml of 7%  $KMnO_4$  and 5 ml of  $HNO_3$  and again evaporate to dryness. Add ca 50 ml of  $H_2O$ , 5 ml of  $HNO_3$ , and 40 ml of saturated soln of  $SO_2$ , and let stand with occasional stirring (breaking up lumps with glass rod) until hydrated oxide of Mn has dissolved.

Filter, wash paper thoroly with  $H_2O$ , add an excess of 10%  $AgNO_3$  to combined filtrate and washings, and boil until  $SO_2$  has been expelled and the  $AgI$  has flocculated. Collect precipitate on weighed Gooch crucible, wash first with  $HNO_3$  (1+99), then twice with  $H_2O$ , dry, and weigh. Calculate as percentage of free I and from result subtract percentage of I found as  $NaI$ , 51. This result is the I organically combined.

53

## TOTAL HALOGENS

*Erythrosine*.—Mix 0.5–1 g of dye with 4 g of  $K_2CO_3$  and moisten to paste with 50% alcohol. Dry, cover with layer of dry  $K_2CO_3$ , and ignite at low red heat. Allow to cool, moisten with few drops of  $H_2O$ , and break up charred mass thoroly. Wash into beaker with ca 20 ml of  $H_2O$ , allow to digest 15 min., and filter. Wash insoluble matter until washings no longer react with  $AgNO_3$ ; then acidify filtrate and washings with  $HNO_3$ , using an excess equivalent to 5 ml of the strong acid, and precipitate halogens with 10%  $AgNO_3$  soln. Collect precipitate on weighed Gooch crucible, wash, dry, and weigh. Compare with sum of results obtained in separate halogen determinations.

54

## SODIUM CARBONATE

*Erythrosine*.—Determine total  $CO_2$  as directed under XVII, 4, using 10 g sample. Calculate and report as  $Na_2CO_3$ .

55

## ORANGE II IN ORANGE I

To a volume of  $H_2O$  soln of sample that contains 1 g of dye add  $H_2O$ , if necessary, to bring volume to 100 ml, and 10 ml of  $HCl$ . Extract this soln by shaking successively in three 500 ml separatory funnels, each containing 100 ml of amyl alcohol and 5 ml of  $HCl$ . Wash each of 3 amyl alcohol extracts by means of six 100 ml por-

tions of  $N \text{ Na}_2\text{CO}_3$  soln (53 g of anhydrous  $\text{Na}_2\text{CO}_3$  to the liter), passed successively thru funnels in the order first used. In washing the acidified amyl alcohol solns, shake gently at first, keeping funnel upright and unstoppered until evolution of  $\text{CO}_2$  is slow enough to permit more vigorous shaking. In same manner wash extracts in second and third funnels with 2 more 100 ml portions of the  $\text{Na}_2\text{CO}_3$  soln and wash extract in third funnel with 2 additional portions of the carbonate soln. Dilute the amyl alcohol solns by adding 350 ml of gasoline (sp. gr. 0.65) to each funnel. Remove dye by extracting completely with requisite number of 10 ml portions of  $\text{H}_2\text{O}$  passed thru funnels, reversing order previously used. Bring volume to 100 or 150 ml by adding  $\text{H}_2\text{O}$ ; add ca 10 g of Na acid tartrate and titrate with standard  $\text{TiCl}_3$  soln, 35. 1 ml of 0.1  $N \text{ TiCl}_3 = 0.008756 \text{ g}$  of orange II.

56

## MARTIUS YELLOW IN NAPHTHOL YELLOW S AND ITS K SALT

Dissolve 5 g of the dye in 150 ml of  $\text{H}_2\text{O}$  (500 ml for the K salt), add 5 ml of  $\text{HCl}$ , and shake vigorously in a separatory funnel 1 min. with 50 ml of gasoline (sp. gr. 0.65). Separate solns and extract aqueous liquid again with 25–30 ml of the solvent. Combine portions of gasoline, decant into clean separatory funnel, and wash with four 25 ml portions of 0.25  $N \text{ HCl}$ . Remove martius yellow by shaking with few portions of 5%  $\text{NaOH}$  soln. Neutralize alkaline dye soln with tartaric acid, add Na tartrate, if necessary, and titrate against standard  $\text{TiCl}_3$  soln as directed under 38(c). 1 ml of 0.1  $N \text{ TiCl}_3 = 0.002134 \text{ g}$  of martius yellow.

Very small quantities (less than 0.1%) may also be determined colorimetrically (in neutral or slightly alkaline soln) by comparison with a standard naphthol yellow S soln, the tinctorial power of which is considered to be 8/10 that of martius yellow.

TARTRAZINE AND AMARANTH<sup>28</sup>

57

## REAGENTS

- (a) *Stannous chloride*.—40%. Add 40 g of  $\text{SnCl}_2$  to sufficient  $\text{HCl}$  to make 100 ml.
- (b) *Ammonia sodium chloride*.—To 12.5 g of  $\text{NaCl}$  and 20 ml of  $\text{NH}_4\text{OH}$  add sufficient  $\text{H}_2\text{O}$  to make 500 ml.
- (c) *Starch iodide paper*.—Triturate 10 parts of starch and 200 parts of  $\text{H}_2\text{O}$ , bring to a boil, and add 1 part of  $\text{KI}$ . Impregnate strips of white filter paper with this soln, dry, and preserve in glass-stoppered bottles.

58

## DETERMINATION

Prepare 1% soln of the mixed dyes. Determine total color as directed in 38 by titrating definite volumes with 0.1  $N \text{ TiCl}_3$ , using Na citrate as buffer. A convenient charge (ca 0.2 g of color) requires ca 10 ml of the standard  $\text{TiCl}_3$ , 35. Make determinations in duplicate.

Pipet definite volume (20 ml if product is pure color) of the dye soln into 250 ml centrifuge bottles and adjust to volume of 50 ml with  $\text{H}_2\text{O}$ . Add exactly 4 ml of the  $\text{SnCl}_2$  soln, mix well, and permit to stand, preferably overnight at room temp. (not below  $20^\circ$ ). The following day place bottles in water bath previously heated to  $50\text{--}60^\circ$ , and maintain contents of bottles at that temp. 5 min. (Since reduction operation has important bearing upon results, the above directions must be observed closely.) Remove bottles from bath and permit to cool to room temp. (Reduced solns should be colorless or very faint yellow.) Add exactly 5 ml of ammonia and mix with contents, which should become slightly alkaline to litmus paper. Centrifuge, and decant thru 15 cm quantitative filter into 400 ml beaker surrounded by ice  $\text{H}_2\text{O}$ . Into beaker measure 15 ml of  $\text{HCl}$  and 0.2 ml of 10%  $\text{CuSO}_4$  soln. Wash residue in bottles thrice with 50 ml portions of the ammonia- $\text{NaCl}$  soln, mix well,

centrifuge each time, and decant thru filter. (Total filtrate in beaker should now measure ca 200 ml.) Cool contents of beaker to 5° and add slowly 1 ml of 10%  $\text{NaNO}_2$  soln. Keep temp. between 5 and 8° for 2 hours, testing with the starch iodide paper at intervals of ca every 30 min. (There should be an excess of nitrous acid at end of this period. Under ordinary conditions quantity of  $\text{NaNO}_2$  stated is found sufficient.) Add 12 ml of 1% dilute alcoholic  $\beta$  naphthol soln to 100 ml of 2  $N$   $\text{Na}_2\text{CO}_3$  in liter beaker and cool to 15°. Into  $\beta$  naphthol soln pour gradually the diazo soln, stirring vigorously. Rinse beaker with some of dye soln and lastly rinse with 25 ml of alcohol and add to dye soln. Heat contents of beaker over steam bath, maintaining temp. of ca 70° for 1 hour, cool, and allow to stand overnight at room temp.

TABLE 4.—Percentage of sulfur, nitrogen, and sodium in permitted food dyes

DYE	SULFUR	NITROGEN	SODIUM	SODIUM SULFATE CORRESPONDING TO SODIUM CON- TENT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Amaranth .....	15.91	4.64	11.41	35.23
Ponceau 3R .....	12.97	5.66	9.30	28.71
Ponceau SX .....	13.35	5.83	9.57	29.55
Erythrosine .....	.....	.....	5.12	15.81
Orange I .....	9.15	8.00	6.57	20.28
Naphthol yellow S .....	8.95	7.82	12.84	39.64
Naphthol yellow S-K salt .....	8.21	7.17	.....	.....
Sunset yellow FCF .....	14.18	6.19	10.17	31.40
Tartrazine:				
Trisodium salt .....	12.00	10.49	12.91	39.85
Disodium salt .....	12.52	10.94	8.98	27.72
Guinea green B <sup>1</sup> .....	9.29	4.06	3.33	10.28
Light green SF yellowish: <sup>1</sup>				
Disodium salt .....	12.13	3.53	5.80	17.90
Monosodium salt .....	12.48	3.64	2.98	9.20
Fast green FCF .....	11.89	3.46	5.69	17.57
Brilliant blue FCF .....	12.13	3.53	5.80	17.91
Indigotine .....	13.75	6.01	9.86	30.42
Yellow AB .....	.....	17.00	.....	.....
Yellow OB .....	.....	16.09	.....	.....
Orange SS .....	.....	10.69	.....	.....
Oil red XO .....	.....	10.15	.....	.....

<sup>1</sup> There is evidence that the disodium salt of guinea green B and the trisodium salt of light green SF yellowish form colorless solutions.

Use six separatory funnels of 250 ml capacity. Measure into each 50 ml of amyl alcohol. Add to first funnel 50 ml of alkaline dye soln. Shake vigorously and wait until sharp separation has occurred; draw off lower layer and pass successively thru the other 5 funnels, and lastly discard lower layer. Repeat procedure with 50 ml portions until entire dye soln is extracted. Rinse beaker with 5–50 ml portions of 0.25  $N$   $\text{HCl}$ , passing them individually thru amyl alcohol extracts, and later discarding. Dilute each amyl alcohol extract with 100 ml of petroleum benzin and extract out orange dye with 100 ml portions of 1/128  $N$   $\text{HCl}$ , shaking vigorously and passing lower extracts thru entire series of funnels. Collect extracts containing orange II in liter casserole. Continue washing amyl alcohol until all orange color is removed. (It will be noted that first funnel will readily give up orange dye inasmuch as the red is not readily washed out at that acid concentration.) Continue acid extraction until each funnel will yield no more orange color.

In presence of large amount of red color, wash last dilute acid extract with mixture of amyl alcohol and petroleum benzin (1:2) in order to remove any red dye that

may have been extracted. Add excess of ammonia (10 ml) to casserole containing the orange color, and evaporate carefully to dryness on steam bath, avoiding spattering. Dissolve coloring matter from casserole with four 25 ml portions of hot  $H_2O$  and transfer to 300 ml Erlenmeyer flask. Rinse casserole with 10 ml of alcohol and add to flask; then add 10 g of Na bitartrate and also 1 drop of 2% light green SF yellowish soln (indicator). Boil vigorously, pass in rapid stream of  $CO_2$ , and titrate with standard  $TiCl_3$  soln, 35, until green color is visible. Note reading and add another drop of standard soln, whereupon green color should be destroyed.

The number of ml of standard  $TiCl_3$  soln required to reduce the orange II = quantity of tartrazine originally present. 1 ml of 0.1 N  $TiCl_3$  = 0.01336 g of tartrazine.

Subtract above titration from original total color titer. Difference is volume necessary to reduce amaranth originally present. 1 ml of 0.1 N  $TiCl_3$  = 0.01511 g of amaranth.

59

## TOTAL NITROGEN

(a) *Guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Proceed as directed under II, 22, using 2 g of sample and a little  $CuSO_4$  to assist oxidation.

(b) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, tartrazine, Yellow AB, yellow OB, naphthol yellow S and its K salt, orange SS, and oil red XO.*—Treat 2 g of the dye with 25 ml of saturated soln of  $SO_2$  and 1 g of Zn dust (for oil-soluble dyes add a few ml of alcohol) and warm mixture gently until colorless (2–3 min.); if it does not become colorless, add more  $SO_2$  soln in small portions at a time until color is destroyed. Then add 30 ml of  $H_2SO_4$  and 0.7 g of  $HgO$  or its equivalent of metallic Hg and digest mixture. Finally make alkaline, distil, and titrate as directed under II, 21, 22, or 23.

## SELECTED REFERENCES

- <sup>1</sup> Schultz, Farbstofftabellen, 5th German ed., 1914.
- <sup>2</sup> Abs. Z. anal. Chem., 24, 625 (1885); 28, 639 (1889); Conn. Agr. Exp. Sta. Rpt. 1899, p. 130.
- <sup>3</sup> J. Am. Chem. Soc., 27, 25 (1905).
- <sup>4</sup> Abs. Z. anal. Chem., 35, 397 (1896).
- <sup>5</sup> U. S. Dept. Agr. Bur. Chem. Bull., 65, p. 152; Ann. fals., 3, 293 (1910); U. S. Dept., Agr. Bur. Chem. Circs., 25 and 63; Abs. Chem. Centr., 69, (2) 943 (1898); J. Ind. Eng. Chem., 8, 614 (1916); 10, 436 (1918).
- <sup>6</sup> U. S. Dept. Agr. Bur. Chem. Bull., 448; U. S. Dept. Agr. Bur. Chem. Circs., 25 and 63; Allen, Commercial Organic Analysis, 4th ed., 1911, Vol. 5; Leach-Winton, Food Inspection and Analysis, 4th ed., 1920; Girard, Analyse des Matières Alimentaires et Recherche de leurs Falsifications, 2nd ed., 1904; U. S. Dept. Agr. Bur. Animal Ind. Circ., 180; J. Ind. Eng. Chem., 8, 1123 (1916); 9, 955 (1917).
- <sup>7</sup> Abs. Z. anal. Chem., 26, 100 (1887); 27, 232 (1888).
- <sup>8</sup> U. S. Dept. Agr. S.R.A., F.D. No. 3, Rev. 1 (1931).
- <sup>9</sup> U. S. Dept. Agr. Bull., 1390, Supplement 1 (1930).
- <sup>10</sup> Abs. Z. anal. Chem., 26, 100 (1887); 27, 232 (1888); Chem. Ztg., 22, 437 (1898); U. S. Dept. Agr. Bur. Chem. Circ., 63; Green, The Identification of Dyestuffs on Animal Fibres, rev. ed., 1913.
- <sup>11</sup> Ber., 21, 3471 (1886).
- <sup>12</sup> U. S. Dept. Agr. Bull., 448, p. 47.
- <sup>13</sup> Formánek, Spektralanalytischer Nachweis künstlicher organischer Farbstoffe, 1900; Formánek and Grandmougin, Untersuchung und Nachweis organischer Farbstoffe auf spektroskopischem Wege, 2nd ed., 1908–13.
- <sup>14</sup> Knecht and Hibbert, New Reduction Methods in Volumetric Analysis, 2nd ed., 1918, p. 72.
- <sup>15</sup> U. S. Dept. Agr. Bur. Chem. Circs., 25 and 63; Allen, Commercial Organic Analysis, 4th ed., 5, 625 (1911); Leach-Winton, Food Inspection and Analysis, 4th ed., 1920.

- <sup>16</sup> Rupe, *Die Chemie der Natürlichen Farbstoffe*, 1900.
- <sup>17</sup> Perkin and Everest, *The Natural Organic Coloring Matters*, 1918.
- <sup>18</sup> Willstätter and Stoll, *Untersuchungen über Chlorophyll, Methoden und Ergebnisse*, 1913.
- <sup>19</sup> Sitz. preuss. Akad. Wiss., **12**, 402 (1914); Ann., **401**, 189 (1913); **408**, 1 (1915); **412**, 164, 195 (1916); Ber. pharm. Ges., **25**, 447 (1915); Ber., **51**, 782, 784 (1918).
- <sup>20</sup> Molisch, Ber. botan. Ges., **14**, 16 (1896).
- <sup>21</sup> Leach-Winton, *Food Inspection and Analysis*, 4th ed., 1920, pp. 161, 558.
- <sup>22</sup> Girard, *Analyse des Matières Alimentaires et Recherche de leurs Falsifications*, 2nd ed., 1904.
- <sup>23</sup> Mass. Inst. Tech. Quart., **21**, 280 (1908).
- <sup>24</sup> J. Assoc. Official Agr. Chem., **5**, 196 (1921).
- <sup>25</sup> U. S. Dept. Agr. Bull., **1390**, Supplement No. 1.
- <sup>26</sup> U. S. Dept. Agr. Bull., **1390**.
- <sup>27</sup> J. Assoc. Official Agr. Chem., **5**, 207 (1921).
- <sup>28</sup> Color Trade J., **13**, 4 (1923).
- <sup>29</sup> J. Assoc. Official Agr. Chem., **15**, 73, 362 (1932).

## XXII. DAIRY PRODUCTS

### MILK

1

#### COLLECTION OF SAMPLE—OFFICIAL

The quantity of sample required depends upon the number of determinations to be made. For the usual analysis collect 250–500 ml ( $\frac{1}{2}$ –1 pint) of sample; for fat determination only, 50–60 ml (ca 2 fl. oz.) will suffice.

In the case of bottled milk collect one or more bottles as prepared for sale. In sampling bulk milk thoroly mix by pouring from one clean vessel into another 3 or 4 times. If this procedure is impracticable, thoroly stir milk at least 30 seconds with a suitable appliance long enough to reach to bottom of container. If cream has formed, continue mixing until all cream is detached from sides of vessel and evenly emulsified thruout liquid.

Place in non-absorbent, air-tight containers and keep cold, but above freezing temp. until examined. When transporting samples by mail, express, or otherwise, completely fill containers, tightly stopper, and mark for identification. A suitable quantity of preservative ( $\text{HgCl}_2$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ , or  $\text{HCHO}$ ) may be used unless presence of preservative is objectionable in connection with physical or chemical tests to be applied in addition to determination of fat.

2

#### PREPARATION OF SAMPLE—OFFICIAL

Before withdrawing portions for analytical determinations, bring sample to 15–20° and mix thoroly by pouring into clean receptacle and back until homogeneous mixture is assured. If lumps of cream do not completely disappear, warm sample to ca 38°, mix thoroly, then cool to 15–20°. In case a measured volume is required in a determination, bring temp. of sample to 20° before pipetting.

3

#### SPECIFIC GRAVITY—TENTATIVE

Determine specific gravity at 15.6/15.6° with a pycnometer or a standard hydrometer.

4

#### ACIDITY—TENTATIVE

Dilute 10–20 ml of milk with an equal volume of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and titrate with standard  $\text{NaOH}$ , using phenolphthalein indicator. Express result as percentage of lactic acid. The determination is conveniently made by measuring 17.6 ml of prepared sample with Babcock pipet, 21(b), diluting with an equal volume of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , which also rinses the pipet, and titrating with 0.1  $N$   $\text{NaOH}$ , using 0.5 ml of phenolphthalein. Number of ml of 0.1  $N$   $\text{NaOH}$  required  $\div 20$  = percentage of lactic acid.

#### CITRIC ACID—TENTATIVE

5

#### PREPARATION OF SAMPLES

To 50 g of milk in 150 ml beaker, add ca 100 mg of tartaric acid and 6 ml of normal  $\text{H}_2\text{SO}_4$  and heat on steam bath for 15 min. Immediately add 3 ml of 20% phosphotungstic acid soln, mix well, and return to steam bath for 5 min. Transfer to 250 ml volumetric flask with alcohol, cool, dilute to mark with alcohol, mix, and filter thru folded paper. Pipet 200 ml of clear filtrate into a centrifuge bottle.



6

## REAGENTS

Use the reagents specified under XXVI, 30.

7

## DETERMINATION

To soln in centrifuge bottle, add 10 ml of the Pb acetate soln, shake vigorously ca 2 min., and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from precipitated Pb salts and test with small quantity of the Pb soln. If precipitate forms, return to centrifuge bottle, add more Pb soln, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow bottle to drain thoroly by inverting several minutes. To the Pb salts in centrifuge bottle add ca 150 ml of H<sub>2</sub>O, shake thoroly, and pass in H<sub>2</sub>S to saturation. Transfer to 250 ml flask, dilute with H<sub>2</sub>O to mark, mix, and filter thru folded paper.

From this point proceed as directed under XXVI, 31, beginning "Pipet 200 ml of filtrate into 500 ml Erlenmeyer flask and evaporate to 75 ml."

Calculate mg of citric acid in portion taken for analysis by following formula:

$$X = 0.695P + 0.028S, \text{ in which}$$

$X$  = mg of citric acid in portion taken for analysis,

$P$  = weight of pentabromacetone (mg); and

$S$  = volume of filtrate (ml).

## TOTAL SOLIDS

8

*Method I.—Official*

Use round flat-bottomed dish not less than 5 cm in diameter, in which may be spread, before weighing, 15–20 g of pure dry sand. Pipet into weighed dish 1.5–3 ml of sample, weigh quickly, and heat on steam bath 30 min., then in oven (preferably vacuum) at temp. of boiling H<sub>2</sub>O to constant weight. Cool in desiccator and weigh quickly to avoid absorption of moisture.

9

*Method II.—(Approximate)—Tentative*

Use the formula:  $0.25L + 1.2F$ , in which  $L$  = lactometer reading at 15.6° (sp. gr. as determined by  $3 \times 1000 - 1000$ ); and  $F$  = percentage of fat in the milk.

10

## ASH—OFFICIAL

Into a weighed dish pipet ca 20 ml of prepared sample, 1, weigh quickly, add 6 ml of HNO<sub>3</sub>, evaporate to dryness, and ignite at a temp. below redness until the ash is free from C. Cool in desiccator, weigh, and report increase in weight as ash.

11

## TOTAL NITROGEN—OFFICIAL

Transfer 5 g of sample to Kjeldahl digestion flask and proceed as directed under II, 21, 22, or 23. Percentage of  $N \times 6.38$  = percentage of "protein."

## CASEIN

(Determination should be made while milk is fresh or nearly so. If not possible to make determination within 24 hours, add 1 part of HCHO to 2500 parts of milk and keep in cool place.)

12

*Method I.—Official*

Place 10 g of sample in beaker with 90 ml of H<sub>2</sub>O at 40–42° and add at once 1.5 ml of acetic acid (1+9). Stir, and let stand 3–5 min. Decant on acid-washed filter, wash by decantation 2 or 3 times with cold H<sub>2</sub>O, and transfer precipitate to filter. Wash once or twice on filter. (Filtrate should be clear, or nearly so.) If first

portions of filtrate are not clear, repeat filtration, and complete washing of precipitate. Determine *N* in washed precipitate and filter paper as directed under II, 21, 22, or 23, and multiply by 6.38 to obtain equivalent of casein.

To sample of milk that has been preserved the acetic acid should be added a few drops at a time, with stirring, and the addition should be continued until the liquid above precipitate becomes clear, or very nearly so.

*Method II.<sup>2</sup>—Tentative*

13

REAGENT

Pipet 250 ml of normal acetic acid into 1000 ml flask. Add 125 ml of normal CO<sub>2</sub>-free NaOH. Make up to 1000 ml with CO<sub>2</sub>-free H<sub>2</sub>O and mix thoroly.

14

DETERMINATION

Pipet 20 ml of sample into 100 ml flask. Add 50 ml of the reagent, mix, make up to volume with H<sub>2</sub>O, and shake well. Set flask in hot H<sub>2</sub>O (50–60°, *not over* 60°) and let stand 15 min. Cool to room temp., add 0.5 g of celite analytical filter aid, shake thoroly, and filter clear thru suitable folded paper, taking care to prevent evaporation during filtration. Determine *N* (A) in 50 ml of the clear filtrate, and determine total *N* (B) in 10 ml of the milk.  $(B - A) \times 6.38$  = the casein in 10 ml of the milk. Report grams of casein per 100 ml of milk, or divide grams per 100 ml by density of milk and report as percentage by weight.

15

ALBUMIN—OFFICIAL

Exactly neutralize filtrate obtained under 12 with 10% NaOH, add 0.3 ml of acetic acid (1 + 9), and heat on steam bath until albumin is completely precipitated. Collect precipitate on acid-washed filter; wash with cold H<sub>2</sub>O; determine *N* as directed under II, 21, 22, or 23, and multiply by 6.38 to obtain equivalent albumin.

LACTOSE

*Optical Method—Official*

16

REAGENTS

(a) *Acid mercuric nitrate soln.*—Dissolve Hg in twice its weight of HNO<sub>3</sub> and dilute with a five-fold volume of H<sub>2</sub>O.

(b) *Mercuric iodide soln.*<sup>3</sup>—Dissolve 33.2 g of KI and 13.5 g of HgCl<sub>2</sub> in 200 ml of glacial acetic acid and 640 ml of H<sub>2</sub>O.

17

DETERMINATION

Determine the specific gravity of the milk as directed under 3. The quantity of sample to be taken varies with the specific gravity and is to be measured at same temp. at which the specific gravity is taken. The volume to be measured will be found in the table, 18, which is based upon twice the normal weight of lactose (32.9 g per 100 ml) for Ventzke sugar scale.

Place quantity of milk indicated under 18 in flask graduated at 102.6 ml. Add 18–20 ml of the acid Hg(NO<sub>3</sub>)<sub>2</sub> soln or 30 ml of the HgI<sub>2</sub> soln, followed by enough 5% phosphotungstic acid soln to make to mark, shake frequently at least 15 min., filter thru dry filter, and polarize. (It is not necessary to heat before polarizing.) If 200 mm tube is used, divide polariscope reading by 2 (or, if 400 mm tube is used, by 4) to obtain percentage of lactose in sample.

18 *Volumes of milk corresponding to a lactose double normal weight<sup>a</sup>*

SPECIFIC GRAVITY OF MILK	VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE)	SPECIFIC GRAVITY OF MILK	VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE)
	<i>ml</i>		<i>ml</i>
1.024	64.25	1.030	63.90
1.025	64.20	1.031	63.80
1.026	64.15	1.032	63.75
1.027	64.05	1.033	63.70
1.028	64.00	1.034	63.65
1.029	63.95	1.035	63.55
		1.036	63.50

## 19

*Gravimetric Method—Official*

Dilute 25 g of sample with 400 ml of H<sub>2</sub>O in 500 ml volumetric flask and add 10 ml of CuSO<sub>4</sub> soln, XXXIV, 32(a), and ca 7.5 ml of a KOH soln of such strength that 1 volume is just sufficient to precipitate completely the Cu as hydroxide from 1 volume of the CuSO<sub>4</sub> soln. (Instead, 8.8 ml of 0.5 N NaOH soln may be used.) After addition of alkali soln, mixture must still have an acid reaction and contain Cu in soln. Fill flask to mark, mix, filter thru dry filter, and determine lactose in aliquot of filtrate as directed under XXXIV, 38. Obtain from Table 9, XLIII, weight of lactose equivalent to weight of Cu<sub>2</sub>O reduced.

## FAT

## 20

*Roose-Gottlieb Method<sup>5</sup>—Official*

Transfer 10 g of the sample to Rührig tube or similar apparatus, add 1.25 ml of NH<sub>4</sub>OH (2 ml if sample is sour) and mix thoroly. Add 10 ml of alcohol and mix well. Add 25 ml of ether, shake vigorously 30 seconds, add 25 ml of petroleum benzin (redistilled slowly at temp. below 65°), and shake again 30 seconds. Let stand 20 min., or until upper liquid is practically clear. Draw off into a suitable flask or metal dish thru small, quick-acting filter as much as possible of ether-fat soln (usually 0.5–0.8 ml will be left). Again extract liquid remaining in tube, this time with 15 ml of each solvent; shake vigorously 30 seconds after each addition; and allow to settle. Draw off clear soln thru the small filter into same flask or dish as before and wash tip of spigot, stopper, funnel, and filter with a few ml of a mixture of the two solvents, in equal parts, free from suspended H<sub>2</sub>O. To insure complete removal of fat, make a third extraction. (This third extraction yields less than 1 mg of fat if previous solns have been drawn off closely.) If flask is used, add a glass bead and evaporate solvents slowly on warm surface; then dry fat in oven at temp. of boiling H<sub>2</sub>O to constant weight. Weigh flask with similar flask as counterpoise. Do not wipe flask immediately before weighing. Remove fat completely with petroleum benzin and dry container with residue. Weigh, and deduct from total weight. Loss in weight = weight of fat. Finally, correct this weight by a blank determination on reagents used.

*Babcock Method<sup>6</sup>—Official*

## 21

## APPARATUS

(a) *Standard Babcock test milk bottle.*—8%, 18-g, 6" milk-test bottle, total height 150–165 mm (5.9–6.5"). Bottom of bottle shall be flat, and axis of neck shall be vertical when bottle stands on level surface. Charge of milk for bottle shall be 18 g.

*Bulb.*—Capacity of bulb to junction with neck shall be not less than 45 ml. Shape of bulb shall be either cylindrical or conical. If cylindrical, outside diameter shall be between 34 and 36 mm; if conical, outside diameter of base shall be between 31 and 33 mm, and maximum diameter between 35 and 37 mm.

*Neck.*—Shall be cylindrical and of uniform diameter from at least 5 mm below lowest graduation mark to at least 5 mm above highest. Top of neck shall be flared to diameter of not less than 10 mm. Graduated portion of the neck shall have a length of not less than 63.5 mm. Total per cent graduation shall be 8. Graduations shall represent whole per cent, 0.5%, and 0.1%, respectively, from 0.0 to 8.0%. Tenths per cent graduations shall be not less than 3 mm in length; 0.5% graduations shall be not less than 4 mm in length and shall project 1 mm to the left; and whole per cent graduations shall extend at least half-way around neck to right and shall project at least 2 mm to left of tenths per cent graduations. Each whole per cent graduation shall be numbered, the number being placed to left of scale. Capacity of neck for each whole per cent on scale shall be 0.20 ml. Maximum error of total graduation or any part thereof shall not exceed volume of smallest unit of graduation.

Each bottle shall be so constructed as to withstand stress to which it will be subjected in centrifuge.

(a) *Testing.*—The Hg and cork, alcohol and buret, and alcohol and brass plunger methods may be used for rapid testing of bottles, but accuracy of any questionable bottle shall be determined by calibration with Hg (13.5471 g of clean, dry Hg at 20° to be equal to 5% on the scale of an 18-g bottle and 10% on the scale of a 9-g bottle), the bottle being previously filled to zero with Hg.

(b) *Pipet.*—The standard milk pipet shall conform to following specifications:

	mm
Total length, not more than.....	330
Outside diameter of suction tube.....	6-8
Length of suction tube.....	130
Outside diameter of delivery tube.....	4.5-5.5
Length of delivery tube.....	100-120
Distance of graduation mark above bulb.....	15-45
Nozzle, straight	
Graduation, to contain 17.6 ml of H <sub>2</sub> O at 20° when bottom of meniscus coincides with mark on the suction tube.	
Delivery, 5-8 seconds.	
Maximum error in graduation, not to exceed 0.05 ml.	
The pipet is to be marked "Holds 17.6 ml."	

(b) *Testing.*—The pipet shall be tested by measuring from a buret the volume of H<sub>2</sub>O (at 20°) which it holds up to the graduation mark.

(c) *Acid measure.*—The device used to measure H<sub>2</sub>SO<sub>4</sub>, whether graduated cylinder or pipet attached to Swedish acid bottle, shall be graduated to deliver 17.5 ml.

(d) *Centrifuge or "tester."*—The standard centrifuge, however driven, shall be constructed thruout and so mounted as to be capable, when filled to capacity, of rotating at necessary speed with minimum of vibration and without liability of causing injury or accident. It shall be heated, electrically or otherwise, to a temp. of at least 55° during the process of centrifuging. It shall be provided with a speed indicator, permanently attached, if possible. Proper rate of rotation may be ascertained by reference to table below. By "diameter of wheel" is meant distance

between inside bottoms of opposite cups measured thru center of rotation of centrifuge wheel while cups are horizontally extended.

Diameter of wheel, inches:	10	12	14	16	18	20	22	24
No. revolutions per minute:	1074	980	909	848	800	759	724	693

(e) *Dividers or calipers*.—For measuring fat column.

(f) *Water bath for test bottles*.—Provided with thermometer and device for maintaining temp. of 55–60°.

## 22

## DETERMINATION

Transfer 18 g of prepared sample, 2, to milk-test bottle by means of the pipet. Blow out milk remaining in pipet tip after free outflow has ceased. Add 17.5 ml of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.82–1.83 at 20°) in small portions and in such a way as to wash all traces of milk into bulb. Temp. of acid shall be 15–20°. Shake until all traces of curd have disappeared; then transfer bottle to centrifuge; counter-balance it; and, after proper speed has been attained, whirl 5 min. Add soft  $\text{H}_2\text{O}$  at 60°, or above, until bulb of bottle is filled. Whirl 2 min. Add hot  $\text{H}_2\text{O}$  until liquid column approaches top graduation of scale. Whirl 1 min. longer at temp. of 55–60°. Transfer bottle to warm water bath maintained at temp. of 55–60°, immerse it to level of top of fat column, and leave until column is in equilibrium and lower fat surface has assumed a final form. Remove bottle from bath, wipe it, and with aid of dividers or calipers measure fat column, in terms of percentage by weight, from its lower surface to highest point of upper meniscus.

The fat column, at time of measurement, should be translucent, of a golden-yellow or amber color, and free from visible suspended particles. Reject all tests in which fat column is milky or shows presence of curd or of charred matter, or in which the reading is indistinct or uncertain.

## ADDED WATER

## 23

*Acetic Serum Method*—Official

(a) *Zeiss immersion refractometer reading*.—To 100 ml of the milk, measured at 20° into a beaker, add 2 ml of 25% acetic acid (sp. gr. 1.035). Cover beaker with watch-glass, place in water bath at 70° for 20 min., then in ice  $\text{H}_2\text{O}$  for 10 min., and separate curd from serum by rapid filtration thru small filter. Transfer portion of clear serum to a refractometer beaker, place in constant temp. bath, and take refractometer reading when temp. of serum has been brought to exactly 20°, as determined by thermometer graduated in tenths of a degree. A reading below 39 indicates added  $\text{H}_2\text{O}$ ; between 39 and 40, the addition of  $\text{H}_2\text{O}$  is suspected. When reading is 40 or below, determine ash in serum as directed under (b).

(b) *Ash*.—Transfer 25 ml of serum to weighed flat-bottomed Pt dish and evaporate to dryness on water bath. Heat over low flame (to avoid spattering) until contents are thoroly charred, place dish in muffle, preferably with pyrometer attached, and ignite to white ash at temp. not greater than 500°. Cool and weigh. Express result as grams per 100 ml. A result below 0.715 g per 100 ml indicates added  $\text{H}_2\text{O}$ . The acetic serum ash  $\times$  factor 1.021 = sour serum ash (dilution of acetic serum being 2%).

## 24

*Sour Serum Method*—Official

(a) *Zeiss immersion refractometer reading*.<sup>8</sup>—Allow milk to become completely sour, filter, and determine immersion refractometer reading of clear serum at 20°. A reading below 38.3 indicates added  $\text{H}_2\text{O}$ .

(b) *Ash*.<sup>9</sup>—Determine ash in 25 ml of serum obtained in (a) as directed under 23(b). A result below 0.730 g per 100 ml indicates added  $\text{H}_2\text{O}$ .

25

*Copper Serum Method<sup>10</sup>—Official*

To 1 volume of  $\text{CuSO}_4$  soln (72.5 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  per liter, adjusted if necessary to read 36 at  $20^\circ$  on scale of Zeiss immersion refractometer, or to sp. gr. of 1.0443 at  $20/4^\circ$ ), add 4 volumes of milk. Shake well and filter. Determine refractometer reading of clear serum at  $20^\circ$ . A reading below 36 indicates added  $\text{H}_2\text{O}$ . When refractometer reading is 36 or below, determine ash of the sour serum as directed under 24(b) or of the acetic serum as directed under 23(b).

*Cryoscopic Method<sup>11</sup>—Official*

26

## APPARATUS

(a) *Cryoscope*.—A cylindrical-shaped Dewar flask of 1 liter capacity and 28 cm internal depth, surrounded by metal casing, is tightly closed by means of large cork of ca 3 cm thickness. Thru center of cork is tightly fitted medium thin-walled glass or metal tube, 250 mm in length by 33 mm outside diameter. At one side of cork is inserted narrow metal inlet tube, lower end of which is formed into perforated loop near bottom of flask. At opposite side is metal tube of T-shape construction and 6 mm internal diameter, intended to afford escape for vapors, and also for introducing volatile fluid into apparatus. At back portion of cork is fitted control thermometer, bulb of which extends nearly to bottom of flask. The freezing test tube is of thin glass, ca 240 mm in length by 29 mm outside diameter, and fits closely into larger tube, which is sealed into cork. In rubber stopper of freezing tube is fitted the standard thermometer. The length of thermometer permits insertion of bulb nearly to bottom of tube and at same time allows complete exposure of scale above stopper. At right side of thermometer a stirring device made of non-corrodible low conductivity metal is fitted into stopper thru short section of thin-walled metal tubing. The lower end extends nearly to bottom of test tube and is provided with horizontal loop encircling thermometer. At left of thermometer is freezing-starter attachment inserted thru opening in stopper formed by means of short section of metal tubing. This device consists of non-corrodible metal rod, at lower end of which is 10 mm length opening for purpose of carrying small fragment of ice. At one side of cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted thru tightly fitting stopper and extending nearly to bottom of large-sized test tube. A short section of glass tubing is inserted thru a second opening in stopper and is connected to vaporizing tube which enters cryoscope. Sulfuric acid is poured into drying tube to level slightly above small inner bulb. At opposite side of apparatus is arranged a drain tube for purpose of

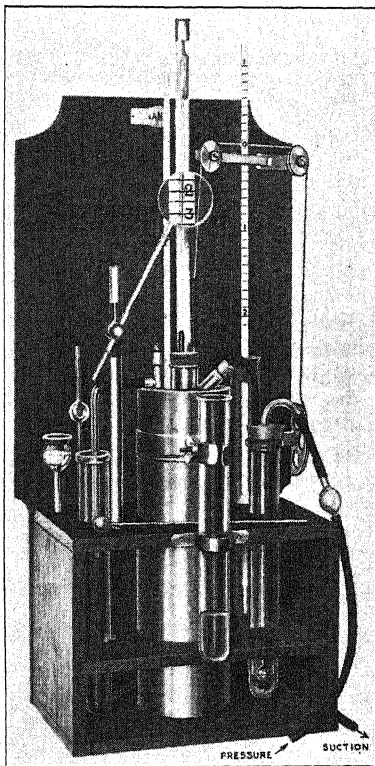


FIG. 27.—HORTVET CRYOSCOPE

conducting vapors away from operator. By means of a pressure and suction pump dry air may be forced into apparatus at a suitable rate and the mixed vapors conducted out thru base of drain tube into sink. An adjustable lens is mounted in a convenient position in front of the thermometer for the purpose of magnifying the scale.

(b) *Standard thermometer*.—A solid-stem instrument having a total length of 58 cm, with a scale portion measuring ca 30 cm. The total scale range is 3°, from +1° to -2°, and each degree division is subdivided into tenths and hundredths. The length of a degree division approximates 1 dm, thus making the smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to 0.001°. Standardize thermometer as directed under 27. Check at frequent intervals, once a week or as often as may be necessary, to keep an accurate record of any changes that may occur.

(c) *Control thermometer*.—A solid-stem instrument ca 58 cm in length and having scale range of +20° to -30°. Test in bath of melting crushed ice for purpose of determining whether 0-mark on scale is correct. Scale graduations should be accurate to within 0.10°.

## 27

## STANDARDIZATION OF THERMOMETER

Make 3 freezing-point determinations by procedure given under 28 on each of following:

(a) *Recently boiled distilled H<sub>2</sub>O*.

(b) *Sucrose soln*.—Dissolve 7 g of pure sucrose in H<sub>2</sub>O and make soln to volume of 100 ml at 20°.

(c) *Sucrose soln*.—Dissolve 10 g of pure sucrose in H<sub>2</sub>O and make soln to volume of 100 ml at 20°.

(A sample of pure sucrose may be obtained from the Director of National Bureau of Standards, Washington, D. C.)

Tabulate the results in the following form:

FREEZING- POINT OBSERVATIONS	PURE WATER	7 GRAMS SUCROSE SOLUTION		10 GRAMS SUCROSE SOLUTION	
		Observed freezing point (-S)	Freezing-point depression S-W (algebraic)	Observed freezing point (-S)	Freezing-point depression S-W (algebraic)
1st					
2nd					
3rd					
Averages	± W	xxxxxxx		xxxxxxx	

Express results as degrees freezing-point depression below average of observed freezing points obtained on sample of pure H<sub>2</sub>O (±W), which may be above (+) or below (-) the 0-mark on scale. Obtain each freezing-point depression of the sucrose solns by algebraic subtraction of average of freezing-point readings of pure H<sub>2</sub>O (±W) from each observed freezing point.

Omit adventitious results, i.e., results that are in marked disagreement with other results obtained by carefully following instructions.

Apply average of freezing-point depressions obtained on standard sucrose solns for correcting thermometer readings obtained on sample of milk in manner illustrated in the tables accompanying Fig. 28.

(Make freezing-point determinations only on samples of milk that show an acidity of not more than 0.18% when determined as directed under 4.)

Insert funnel-tube into vertical portion of T-tube at one side of apparatus and pour in 400 ml of ether previously cooled to 10° or lower. Close vertical tube by means of small cork and connect pressure pump to inlet tube of air-drying attachment. Adjust pump so as to pass air thru apparatus at moderate rate, as may be judged by agitation of the  $\text{H}_2\text{SO}_4$  in the drying tube. Continuous vaporization of the ether will cause lowering of temp. in flask from ordinary room temp. to 0° in 5–10 min. Continue temp. lowering until control thermometer registers near -3°. At this stage, by lowering gage tube into ether bath, then closing top by means of forefinger and raising to suitable height, an estimate can be made as to quantity of ether necessary to pour in for purpose of restoring the 400 ml volume. When volume of ether has been adjusted to 400 ml an additional 10–15 ml is sufficient on an average for each succeeding determination. Pour into freezing test tube sufficient  $\text{H}_2\text{O}$  (30–35 ml), boiled and cooled to 10° or lower, to submerge thermometer bulb. Insert thermometer together with stirrer and lower test tube into larger tube. A small quantity of alcohol, sufficient to fill lower space between the 2 test tubes, will serve to complete the conduction medium between freezing bath and liquid to be tested. Keep stirrer in steady up-and-down motion at rate of approximately one stroke each 1 or 2 seconds, or even at slower rate, provided cooling proceeds satisfactorily. Maintain passage of air thru apparatus until temp. of cooling-bath reaches -2.5°, at which time the top of the Hg thread in thermometer usually recedes to position near freezing point of  $\text{H}_2\text{O}$ . Maintain temp. of cooling-bath at -2.5° and continue manipulation of stirrer until a super-cooling of sample of 1.0–1.2° is observed. As a rule, at this time liquid will begin to freeze, as may be noted by rapid rise of the Hg. Manipulate stirrer slowly and carefully 3 or 4 times as Hg column approaches its highest point. By means of a suitable light-weight cork mallet tap upper end of thermometer cautiously a number of times until top of the Hg column remains stationary at least 1 min. Observe exact reading on thermometer scale, taking necessary precautions to avoid parallax, and estimate to 0.001°. When the observation has been satisfactorily completed, make a duplicate determination; then remove thermometer and stirrer and empty  $\text{H}_2\text{O}$  from freezing tube.

Rinse tube with ca 25 ml of sample of milk, cooled to 10° or lower; measure into tube 30–35 ml of milk or enough to submerge thermometer bulb; and insert tube into apparatus. Maintain temp. of cooling-bath at 2.5° below probable freezing point of sample. Make determination on the milk, following same procedure as that used in determining freezing point of  $\text{H}_2\text{O}$ . As a rule, however, it is necessary to start freezing action in milk by inserting the freezing starter (kept in contact with ice for several minutes, and in the open end of which has been wedged a fragment of ice) at time when Hg column has receded to 1.0–1.2° below probable freezing point. A rapid rise of the Hg results almost immediately. Remove starter and manipulate stirrer slowly and carefully 2 or 3 times while Hg approaches its highest point. Complete adjustment of the Hg column in same manner as in preceding determination; then, avoiding parallax, observe exact reading on thermometer scale and estimate to 0.001°. *The algebraic difference* between the average of readings obtained on the  $\text{H}_2\text{O}$  and the reading obtained on the sample of milk represents *freezing-point depression* of the milk. To determine the true *freezing-point* ( $T^1$ ) of the milk, subtract from the freezing-point depression, the freezing-point depression of 7% sucrose soln as determined by the laboratory thermometer. Multiply difference by the correction factor for the thermometer. Add to product 0.422 (freezing point of 7% sucrose soln by Bur. Standards thermometer). See example under Fig. 28.



Two Bureau of Standards tested thermometers gave intervals of  $0.199^\circ$  and  $0.200^\circ$ , respectively, between the freezing-point depression readings of the two sucrose solns. One thermometer (Fig. 28) gave freezing-point depressions  $-0.422^\circ$  ( $+0.079$  and  $-0.343$ ) and  $-0.621^\circ$  ( $+0.079$  and  $-0.542$ ), respectively, for the two sucrose solns, while the other gave  $-0.422^\circ$  and  $-0.622^\circ$ , respectively.

Laboratory Thermometer No. 2.

	7 GRAMS SUCROSE TO 100 ML	10 GRAMS SUCROSE TO 100 ML
WATER		
Av. $+0.056^\circ$	$-0.425^\circ$	$-0.621^\circ$

Interval =  $0.196$   
 $0.196$  equiv.  $0.199$   
 Correction factor =  $1.015$

Laboratory Thermometer No. 24.

	7 GRAMS SUCROSE TO 100 ML	10 GRAMS SUCROSE TO 100 ML
WATER		
Av. $0.000^\circ$	$-0.420^\circ$	$-0.625^\circ$

Interval =  $0.205$   
 $0.205$  equiv.  $0.199$   
 Correction factor =  $0.971$

*Example*

Laboratory Thermometer No. 24.  
 F. pt. Depression Sample Milk =  $0.548$   
 $(0.548 - .420) 0.971 = 0.124$   
 True freezing point =  $0.422 + 0.124$   
 $(= 0.546^\circ \text{ below zero C})$

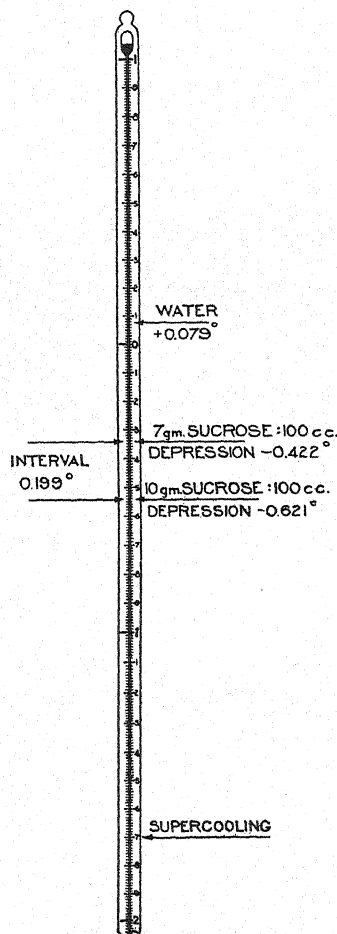


FIG. 28.—U. S. BUREAU OF STANDARDS TESTED THERMOMETER

Ascertain percentage of added  $H_2O$  corresponding to determined freezing-point depression from Table 22, XLIII. The percentage of added  $H_2O$  ( $W$ ) may also be calculated as follows:

$$W = \frac{100 (T - T^1)}{T}, \text{ in which}$$

$T$  = average freezing point of normal milk ( $-0.550^\circ$ ), and  
 $T^1$  = true freezing point on given sample.

A tolerance of 3% may be allowed on results for added  $H_2O$  determined on basis of average freezing-point depression of  $-0.550^\circ$ . Owing to narrow variations found in market milks of genuine character it is not necessary to deduct tolerance figure from results showing added  $H_2O$  in excess of 3%.

GELATIN<sup>12</sup>

29

*Qualitative Test—Official*

To 10 ml of the milk add an equal volume of acid  $Hg(NO_3)_2$  soln ( $Hg$  dissolved in twice its weight of  $HNO_3$  and this soln diluted to 25 times its volume with  $H_2O$ ), shake mixture, add 20 ml of  $H_2O$ , shake again, allow to stand 5 min., and filter. If much gelatin is present, filtrate will be opalescent and cannot be obtained quite clear. To portion of filtrate in a test tube add an equal volume of saturated aqueous picric acid soln. A yellow precipitate will be produced in presence of any considerable quantity of gelatin, while smaller quantities will be indicated by cloudiness.

NOTE: In applying this test to sour, fermented, cultured, or very old samples of milk, cream, or buttermilk; to sterilized cream or evaporated milk; or to cottage cheese, use care to recognize precipitates produced by picric acid when added to the  $Hg(NO_3)_2$  filtrates from these materials in the absence of gelatin. Such samples, with or without rennet and entirely free from gelatin, give, on standing, distinct precipitates when treated as above outlined. In every case, however, these precipitates differ in character from those produced by picric acid with gelatin. The gelatin-picric acid precipitate is finely divided, more apt to remain in suspension, settles only slowly, and adheres tenaciously to sides and bottom of container from which it is rinsed with difficulty. Precipitates produced by picric acid in the absence of gelatin are flocculent, separate readily (leaving serum practically clear), do not adhere to walls of container, and are easily removed by rinsing with  $H_2O$ . When gelatin is present in sample, the gelatin-picric acid precipitate will remain in suspension long after the flocculent precipitate has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to bottom and sides of test vessel. If gelatin is present in relatively high concentration (1%), the gelatin-picric acid precipitate will be voluminous and will settle rather quickly.

30

## PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII. To test for salicylic acid or benzoic acid acidify 100 ml of the milk with 5 ml of  $HCl$  (1+3), shake until curdled, filter, and treat clear filtrate as directed under XXXII, 2, 3, or 6.

To test for  $HCHO$  proceed as directed under XXXII, 19-25, inclusive, applying test directly to the milk.

31

COLORING MATTERS<sup>12</sup>—OFFICIAL

Warm ca 150 ml of milk in casserole over flame, add ca 5 ml of acetic acid (1+3), and slowly continue heating nearly to boiling point while stirring. Gather the curd, when possible, into one mass with stirring rod and pour off the whey. If curd breaks up into small flecks, separate from whey by straining thru sieve or colander. Press curd free from adhering liquid, transfer to small flask, macerate with ca 50 ml of ether, keeping flask tightly corked and shaking at intervals, and allow to stand for several hours, preferably overnight. Decant the ether extract into evaporating dish, remove ether by evaporation, and test fatty residue for annatto as directed under XXI, 16(b).

The curd of an uncolored milk is perfectly white after complete extraction with ether, as is also that of a milk colored with annatto. If extracted fat-free curd is distinctly orange or yellowish in color, a coal tar dye is indicated. In many cases if a lump of a fat-free curd in a test tube is treated with a little  $HCl$  the color changes to pink, indicating presence of a dye similar to aniline yellow or butter yellow or

perhaps one of the acid azo yellows or oranges. In such cases, separate and identify coloring matter present in the curd as directed under **XXI, 11**. If aniline yellow, butter yellow, or any other oil-soluble dye is present, the greater part will be found in the ether extract containing the fat. In such cases proceed as directed under **XXI, 3**.

In some cases the presence of coal tar dyes can be detected by treating ca 100 ml of milk directly with equal volume of HCl in porcelain casserole, giving dish a slight rotary motion. In presence of some dyes the separated curd acquires a pink coloration.

#### SEDIMENT TEST<sup>12</sup>

(Taken from standard Methods of Milk Analysis, American Public Health Association. This method has been edited to conform in part to the style of this publication.)

#### 32

##### SAMPLING

Pint samples only are regarded as standard. If quart or any other size of sample is used, report the size. Take samples from well-stirred cans or vats of milk or from pint or well-shaken quart bottles of milk. Do not take samples from unstirred bottom milk of 40 quart or other cans. Measure quantity of milk used with reasonable accuracy.

#### 33

##### PREPARATION OF SEDIMENT DISKS

Strain the pint sample of milk thru suitable sediment tester fitted with firm cotton disk (type furnished by Lorenz Model Co., Madison, Wis., is suitable) placed over opening 1" in diameter. (It will hasten process of filtering if provision is made for warming the milk, or it may be forced thru disk by air pressure.)

#### 34

##### PREPARATION OF STANDARD SEDIMENT DISKS

Dry a quantity of weathered cow dung in oven and grind in mill until most of it will pass 100-mesh and practically all will pass 60-mesh screen. Accurately weigh 0.1 g and transfer to a 1000 ml flask, using 50% sugar soln to wash all fine particles down into flask. Make volume up to mark with more of the sugar soln after most of fine particles have been wetted by shaking the half-filled flask thoroly several times. After volume is made up to mark, shake contents of flask vigorously every 5 min. for sufficient time to saturate particles thoroly ( $\frac{1}{2}$ -1 hr.). When particles have been thoroly wetted it will be noted that the sugar soln will hold them evenly in suspension, and mixture is ready to use in making the standard disks.

On the basis of 0.1 g per 1000 ml, 10 ml of the sugar soln contains 1 mg of sediment. Make test disks with one of the usual sediment testers, using varying volumes of the sediment suspension. Place several ounces of filtered skimmed milk in sediment tester and add varying volumes of the sediment suspension. After forcing milk thru disks, run thru small quantity of filtered skimmed milk to obtain a more even distribution of the sediment on the disk.

Remove disk from the tester, mount permanently on a stiff paper, allow to dry, and then make permanent by spraying with a strong disinfectant such as corrosive sublimate. A good apparatus for this purpose is an ordinary throat atomizer, provided caution is observed not to use corrosive sublimate in contact with metal. Below each mounted standard disk on the paper note quantity of dried material that the dirt or filth on the disk represents.

NOTE: An excellent set of Standard disks has been prepared and photographed by the Connecticut State Department of Health Laboratory. Thru the courtesy of

the Connecticut laboratory, photographic copies of these standard disks may be secured thru the office of the American Public Health Association, 50 West 50th St., New York City.

The standards given, based on pint samples of milk to which weighed amounts of sediment have been added, cover the entire range from "clean" to "very dirty" milk. Numerical ratings are given for the convenience of those who wish to use these standard disks as the basis of percentage or other numerical scores. No attempt should be made to grade as sediment any hair, piece of hay or straw, or any large particle of dirt. These should be reported separately.

#### PHOSPHATASE TEST FOR PASTEURIZATION<sup>14</sup> TENTATIVE

35

##### COLLECTION OF SAMPLE

Proceed as directed under 1, except to be assured that no preservative is present and not more than 48 hours has elapsed between the time of sampling and receipt at laboratory. If samples are refrigerated, observe precautions to prevent freezing.

36

##### REAGENTS

(a) *Buffer substrate*.—Dissolve 1.09 g of disodium phenyl phosphate and 11.54 g of sodium veronal (sodium diethyl barbiturate) in  $H_2O$  saturated with  $CHCl_3$  and dilute to 1 liter. Add 10 ml of  $CHCl_3$  per liter and store reagent in refrigerator.

(b) *Folin-Ciocalteu phenol reagent*.—Dissolve 100 g of sodium tungstate ( $Na_2WO_4 \cdot 2H_2O$ ), (according to Folin) and 25 g of sodium molybdate ( $Na_2MoO_4 \cdot 2H_2O$ ) in 700 ml of  $H_2O$  in 1500 ml flask connected by ground-glass joint to reflux condenser. Add 50 ml of 85% phosphoric acid and 100 ml of  $HCl$  and reflux gently for 10 hours. Cool and add 150 g of  $Li_2SO_4$ , 50 ml of  $H_2O$ , and 4–6 drops of liquid Br. Boil mixture without condenser 15 min. to remove excess Br. Cool, transfer to 1 liter flask, dilute to volume with  $H_2O$ , and filter. (Finished reagent should have golden yellow color; reject it if it has a greenish tint.) Keep reagent in refrigerator, protected from dust. For use dilute 1 volume of this stock reagent with 2 volumes of  $H_2O$ .

(c) *Sodium carbonate soln*.—Prepare a 14% or 1.32 *M* soln of anhydrous  $Na_2CO_3$ .

(d) *Filter paper*.—Whatman No. 40, 11 cm. diam. (Use only this grade as interfering substances are at times extracted from other grades.)

37

##### PERMANENT PHENOL STANDARDS

(a) *Color soln, grey*.—Dissolve in  $H_2O$ , 31.9 g of Co chloride ( $CoCl_2 \cdot 6H_2O$ ), 67.5 g of Cu sulfate ( $CuSO_4 \cdot 5H_2O$ ), and 75 g of Ni sulfate ( $NiSO_4 \cdot 6H_2O$ ). Add 32 ml of  $HCl$  and 45 ml of  $H_2SO_4$  and dilute to 500 ml.

(b) *Color soln, red*.—Dissolve 476 g of Co chloride ( $CoCl_2 \cdot 6H_2O$ ) in  $H_2O$  and filter. To filtrate add 100 ml of  $HCl$  and dilute to 1 liter.

(c) *Color soln, blue*.—Dissolve 300 g of Cu sulfate ( $CuSO_4 \cdot 5H_2O$ ) in  $H_2O$ , add 20 ml of  $H_2SO_4$ , and dilute to 1 liter. (Should crystals appear when soln is cooled to below 20°, warm slightly before using to insure complete solubility.)

Prepare permanent color standards equivalent to phenol concentrations of from 0.01 to 0.15 mg per 0.5 ml of sample by combining the quantities of color solns, a, b, and c indicated in table and diluting to 10 ml with  $H_2O$  in each case; e.g., 0.3 ml of soln a + 0.106 ml of soln b + 0.96 ml of soln c +  $H_2O$  to make a volume of 10 ml is equivalent to a phenol concentration of 0.01 mg in 0.5 ml of sample.

These color standards are suitable for use only in natural daylight. If, however, a turquoise blue, unglazed, opaque glass plate is used to deflect the light from a daylight lamp thru the tubes of standards and sample, accurate color comparisons can be made in the absence of daylight. Since the standards are prepared for use only

at 13 mm depth of color, tubes of different diameter can not be used for accurate work.

38

*Preparation of permanent phenol standards*

PHENOL	COLOR SOLUTION		
	GREY (a)	RED (b)	BLUE (c)
<i>mg/0.5 ml</i>	<i>ml</i>	<i>ml</i>	<i>ml</i>
0.01	0.30	0.106	0.96
0.02	0.40	0.140	1.16
0.03	0.55	0.180	1.65
0.04	0.65	0.216	2.10
0.06	0.92	0.286	3.00
0.09	1.30	0.326	4.40
0.12	1.70	0.360	5.70
0.15	2.50	0.396	7.10

39

## DETERMINATION

Transfer 10 ml of the buffer substrate soln into test tube 20 mm×160 mm and add 0.5 ml of the milk to be tested. Add a few drops of  $\text{CHCl}_3$ , mix thoroly by rotating tube, and cover to protect contents from dust. (Do not use rubber or cork stoppers; paper toweling placed over open end of tube is satisfactory.) Warm to 37–39° in water bath and incubate at 34–37° for not less than 18 and not more than 24 hours. After incubation add 4.5 ml of the diluted Folin-Ciocalteu reagent. Mix, and allow to stand 3 min. Filter, and transfer 5 ml of filtrate to test tube of 13 mm diam. Add 1 ml of the  $\text{Na}_2\text{CO}_3$  soln and mix thoroly by rotating tube. Place tube in boiling water bath for 5 min. and filter. Cool, and estimate color of filtrate by comparison with the permanent color standards.

40

## CONTROL TEST

(To check deterioration of reagents and presence of interfering substances in sample.)

To 10 ml of the buffer substrate soln, add 4.5 ml of the Folin-Ciocalteu reagent and 0.5 ml of the milk sample. (Do not incubate.) Mix thoroly, allow to stand 3 min., and filter. To 5 ml of filtrate add 1 ml of the  $\text{Na}_2\text{CO}_3$  soln, mix thoroly by rotating tube, heat in boiling water bath 5 min., and filter. Cool, and compare color of filtrate with the permanent color standards. If the phenol value obtained is greater than 0.02 mg, subtract excess from the phenol value of the incubated sample to obtain the phenol value indicative of pasteurization treatment.

A phenol value of 0.04 mg of phenol per 0.5 ml of sample generally indicates milk heated to 143°F. for 30 min. A value greater than this indicates progressively inadequate heat treatment. In reporting results, give the mg of phenol per 0.5 ml of sample as well as an interpretation as to whether the milk is pasteurized or under-pasteurized.

## VITAMIN D MILK

41

## PRESERVATION OF SAMPLE

The sample of the fluid milk to be assayed shall be delivered to the assayer in its original container immediately after collection or shall be stored under refrigeration in an iced container until delivered. After delivery to the assayer, the milk shall be preserved in its homogeneous state by refrigeration at a temp. of not more than 10°C (50°F) for a period of not more than 10 days, or the sample shall be preserved

for not more than 30 days by the addition of 2 drops of 10% formalin to one quart of milk in addition to refrigeration at a temp. of not more than 10°C (50°F). Evaporated and reconstituted milk shall be preserved in the same manner as fluid milk. A sample soured or curdled is unsuitable for assay purposes. A sample of dried milk, after being opened by the assayer, shall be preserved by refrigeration at a temp. of not more than 10°C (50°F).

42

## PRELIMINARY PERIOD

Thruout the preliminary period each rat shall be raised under the immediate supervision of, or according to directions specified by, the assayer. Throughout the preliminary period the rats shall be maintained on a dietary regimen that shall provide for normal development in all respects, except that the supply of vitamin D shall be limited to such a degree that rats, weighing between 40 and 60 g at an age of 21-30 days, and subsisting for an interval of 18-25 days on a suitable rachitogenic diet, shall manifest evidence of severe rickets.

43

## DEPLETION PERIOD

A rat shall be suitable for the depletion period when the age of rat does not exceed 30 days, and if body weight of the rat exceeds 44 g, and does not exceed 60 g, and if animal manifests no evidence of injury, or disease, or anatomical abnormality that might hinder growth and development. Thruout the depletion period each rat shall be provided with rachitogenic diet and distilled water or water U.S.P. ad libitum, and during this period no other dietary supplement shall be available to the animal.

44

## ASSEMBLING RATS INTO GROUPS FOR ASSAY PERIOD

Rats that are suitable for the assay period shall be assembled into groups. For each assay milk there shall be one or more assay groups. In the assay of one assay milk there shall be provided at least one reference group, but one reference group may be used for concurrent assay of more than one assay milk. On any one day during the interval of assembling rats into groups, the total number of rats that shall have been assigned to make up any one group shall not exceed by more than 2 the number of rats that shall have been assigned to make up any other group. When the assembling of all groups shall have been completed, total number of rats in each group shall be the same. Not more than 3 rats from 1 litter shall be assigned to the assay group unless an equal number of rats from the same litter are assigned to the reference group. There shall be a sufficient number of animals in each group to meet the requirements specified under 48.

45

## ASSAY PERIOD

A rat shall be suitable for the assay period, provided that depletion period shall have exceeded 18 days and shall not have exceeded 25 days, and provided that a rat shall manifest evidence of rickets characterized by a distinctive, wobbly, rachitic gait and by enlarged joints. The presence of rickets may also be established by examination of a leg bone of one member of a litter by the "line test," 46, or by x-ray examination of the animals selected for the assay. Each rat shall be kept in an individual cage and shall be provided with the rachitogenic diet and distilled water or water U.S.P. ad libitum. On any calendar day of the assay period the assay and reference groups shall receive a rachitogenic diet compounded from the same lots of ingredients. Thruout the first 6 days of the assay period each rat in any one assay group shall be fed daily a dose of the assay milk, and thruout the first 6 days of the assay period each rat in any one reference group shall be fed daily a dose of

reference oil and in addition, and separate from the rachitogenic diet, an amount of ether-extracted skim milk powder equal in weight to the solids-not-fat contained in the daily dose of assay milk fed the assay group, except that the following deviation from the daily feeding may be permissible: that the daily dose of milk or reference oil plus ether-extracted skim milk powder may be doubled on the day preceding a one-day holiday. During the remainder of the assay period, neither the assay milk nor the reference oil plus ether-extracted skim milk powder shall be fed. The following optional methods of feeding the assay milk and the reference oil plus ether-extracted skim milk powder shall be permissible, but both the assay milk and the reference oil plus ether-extracted skim milk powder shall be fed according to the same method: the supplements may be fed on the first day of the assay period or in equal portions on the first, third, and fifth days of a 7-day or 10-day assay period or on the first 8 days of a 10-day assay period; the supplements may be fed admixed with a quantity of the basal ration that will be consumed within the first 5 days of a 7-day assay period or within the first 8 days of a 10-day assay period. In each case the unsupplemented basal ration shall be made available during the remainder of the assay period. The quantity of reference oil to be fed shall be that found by experience to cause an extent and degree of calcification of the rachitic metaphysis equal to or greater than a condition described as positive macroscopic evidence of calcification, but less than an extent and degree of calcification described as complete healing. When a vitamin D milk is to be assayed to determine whether the vitamin D potency is that claimed, the quantity of assay milk fed shall be that calculated to contain the same number of units of vitamin D as contained in the quantity of reference oil fed. The quantity of ether-extracted skim milk powder to be fed shall be calculated on the basis of 9% solids-not-fat in whole milk. At the termination of the assay period each rat shall be killed and one or more leg bones examined for the healing of the rachitic metaphysis according to the "line test,"

46. The reference oil may be diluted before being fed with an edible vegetable oil free from vitamins A and D. The diluted oil shall be stored in the dark at a temp. not exceeding 10°C (50°F), the storage period not to exceed 30 days. Not more than 1/10 ml of the diluted oil shall be fed as a daily dose. The ether-extracted skim milk powder may be fed as a dry powder or it may be reconstituted with distilled water to liquid skim milk. During the assay period all conditions of environment (particularly with reference to physiologically active radiations) shall be maintained as uniformly as possible with respect to the assay and reference groups.

## 46

## LINE TEST

The line test shall be made on the proximal end of a tibia or distal end of a radius or ulna. The end of the desired bone is removed from the animal and cleaned of adhering tissue. A longitudinal median section shall be made thru the end of the bone with a clean, sharp blade to expose a plane surface thru the junction of the epiphysis and diaphysis. In any one assay the same bone of all the animals must be used and sectioned thru the same plane. Both sections of the bone shall be rinsed in distilled H<sub>2</sub>O and shall then be immersed in a 2% aqueous soln of AgNO<sub>3</sub> for 1 min. The sections shall then be rinsed in distilled H<sub>2</sub>O and the sectioned surfaces of the bone shall be exposed in H<sub>2</sub>O to daylight or other source of actinic light until the calcified areas have developed a clearly defined stain without marked discoloration of the uncalcified areas. Records shall be made immediately of the extent and degree of calcification of the rachitic metaphysis of every section. It shall be permissible to use modifications of the described procedure for staining, provided that such modified procedures clearly differentiate between calcified and uncalcified areas. The

assayer shall make a distinction between staining due to congestion in the rachitic metaphysis and healing as clearly indicated by the presence of calcium salts stained with silver.

47

## RECORDING OF DATA

On the day beginning the assay period and on the 7th or 10th day thereafter, depending on the duration of the assay period, a record shall be made of the body weight of each rat. A record shall be made of the quantity of rachitogenic diet consumed per rat during the assay period. Numerical values shall be assigned to the extent and degree of calcification of the rachitic metaphysis of the bones examined by the line test so that it will be possible to average the performance of each group.

48

## POTENCY OF THE ASSAY MILK

The data from a reference group shall be considered valid for establishing the vitamin D potency of the assay milk only when two-thirds or more, but not less than 7 rats, show individually an extent and degree of calcification of the rachitic metaphysis equal to or greater than a condition described as positive macroscopic evidence of calcification, but less than an extent and degree of calcification described as complete healing. When the average response of the assay group (in which two-thirds or more, but not less than 7 rats, show individually an extent and degree of calcification described as positive macroscopic evidence of healing) is equal to or greater than that of the reference group, the vitamin D content of the milk fed during the assay period is equal to or greater than the vitamin D content of the reference oil fed during the assay period. When the average response of the assay group is less than that of the reference group, the vitamin D content of the milk fed during the assay period is less than that of the reference oil fed during the assay period. The data from a rat shall be considered valid for establishing the average performance of a group only on the condition that the weight of the rat at the termination of the assay period, shall equal or exceed the weight of the rat on the beginning day of the assay period and that the rat has consumed an average of not less than 4 g of rachitogenic diet daily during the assay period and on the condition that the rat has consumed each prescribed dose of assay milk within 24 hours from the time it was fed. When the above conditions have been met and the response of the assay group is equal to that of the reference group the vitamin D potency of the assay milk may be calculated as follows:

49

## CALCULATION

Let  $R$  = total number of U.S.P. units of vitamin D fed each rat in the reference group during the assay period.

Let  $M$  = total number of ml of assay milk fed each rat in the assay group during the assay period.

946 = ml in 1 quart.

Then  $R \times \frac{946}{M}$  = U.S.P. units of vitamin D per quart.

50

## DEFINITIONS

The term *assay group* means a group of rats to which the assay milk (vitamin D milk) shall be administered during the assay period. The term *assay milk* means the milk (vitamin D milk) under examination for its vitamin potency. The term *assay*



*period* means the interval in the life of a rat between the last day of the depletion period and the eighth or eleventh day thereafter. The term *assemble* means the procedure by which rats are selected and assigned to groups for the purpose of feeding, care, and observation. The term *daily* means each of the first 6 or 8 days of the assay period. The term *depletion period* means the interval in the life of a rat between the last day of the preliminary period and the first day of the assay period. The term *dose* means the quantity of reference oil or of assay milk or other supplement to be fed daily to a rat during an assay period. The term *fed* means made readily available to the rat or administered to the rat by mouth. The term *ground gluten* means the clean, sound product made from wheat flour by the almost complete removal of starch, and contains not more than 10% of moisture, and, calculated on the water-free basis, not less than 14.2% of nitrogen, not less than 15% of nitrogen-free extract (using the protein factor 5.7), and not more than 5.5% of starch (as determined by the diastase method), XXVII, 31. The term *group* means 7 or more rats maintained on the same required dietary regimen during the assay period. The term *preliminary period* means the interval in the life of a rat between the seventh day after birth and the first day of the depletion period. The term *reference oil* means The United States Pharmacopoeial Reference Cod Liver Oil distributed by the Board of Trustees of the United States Pharmacopoeial Convention. The term *rachitogenic diet* means the uniform mixture of the food material, and in the proportions named, in either of the following formulas:

51

*Rachitogenic Diet No. 1*

	<i>per cent</i>
Whole yellow maize, ground	33
Whole wheat, ground	33
Ground gluten	15
Gelatin	15
Calcium carbonate ( $\text{CaCO}_3$ )	3
Sodium chloride ( $\text{NaCl}$ )	1

52

*Rachitogenic Diet No. 2*

	<i>per cent</i>
Whole yellow maize, ground	76
Ground gluten	20
Calcium carbonate ( $\text{CaCO}_3$ )	3
Sodium chloride ( $\text{NaCl}$ )	1

## CREAM

53

## COLLECTION OF SAMPLE—OFFICIAL

Proceed as directed under 1. Analyze sample as soon as practicable, preferably not later than 3 days after taking.

54

## PREPARATION OF SAMPLE—OFFICIAL

Immediately before withdrawing portions for the determinations, mix sample by shaking, pouring, or stirring until it pours readily and a uniform emulsion has been secured. If sample is very thick, warm it to 30–35°, and mix. In case lumps of butter have separated, heat sample to 38° or, if necessary, to 50°, by placing in warm water bath. Thoroughly mix portions for analysis and weigh immediately. (In commercial testing for fat by the Babcock method, it may be advisable to warm all samples to

38–50° in water bath previous to mixing.) Avoid overheating sample, thereby causing cream to “oil off” (especially necessary in case of thin cream).

55 TOTAL SOLIDS—OFFICIAL

Proceed as directed under 8, using 2–3 g of the sample.

56 ADDED WATER<sup>14</sup>—OFFICIAL

Proceed as directed under 26, but use the following formula to calculate percentage of added H<sub>2</sub>O:

$$W = \frac{\% \text{ Serum in Cream } (T - T')}{T}, \text{ in which}$$

$W$  = percentage of added H<sub>2</sub>O;

$T$  = freezing point of undiluted cream (–0.550°);

$T'$  = observed freezing point of given sample; and

$\% \text{ Serum} = 100\% - (\% \text{ fat} + \% \text{ protein})$ .

If protein is not determined it may be assumed to be 38% of solids-not-fat.

57 ASH—OFFICIAL.—See 10.

58 TOTAL NITROGEN—OFFICIAL.—See 11.

LACTOSE

59 *Gravimetric Method—Official.—See 19.*

FAT

60 *Roese-Gottlieb Method—Official*

Transfer 5 g of sample to Röhrig tube or similar apparatus, dilute with H<sub>2</sub>O to ca 10.5 ml, and proceed as directed under 20, beginning “add 1.25 ml of NH<sub>4</sub>OH.

*Babcock Method<sup>8</sup>—Official*

61 APPARATUS

(a) *Test bottles.*—Standard Babcock test bottles for cream shall be as follows:

(1) 50%, 9-g, short-necked, 6" cream-test bottle.—Total height 150–165 mm (5.9–6.5"). The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when bottle stands on level surface. The charge of cream for bottle shall be 9 g.

*Bulb.*—The capacity of bulb to junction with neck shall be not less than 45 ml. The shape of bulb shall be either cylindrical or conical. If cylindrical, outside diameter shall be 34–36 mm; if conical, outside diameter of base shall be 31–33 mm, and maximum diameter, 35–37 mm.

*Neck.*—The neck shall be cylindrical and of uniform diameter from at least 5 mm below lowest graduation mark to at least 5 mm above highest. The top of neck shall be flared to diameter of not less than 15 mm. The graduated portion of neck shall have a length of not less than 63.5 mm. The total per cent graduation shall be 50. The graduations shall represent 5, 1, and  $\frac{1}{2}\%$ , respectively, from 0.0 to 50%. The 5% graduations shall extend at least half-way around neck to right; the  $\frac{1}{2}\%$  graduations shall be not less than 3 mm in length; and the 1% graduations shall be intermediate in length between the 5% and  $\frac{1}{2}\%$  graduations and shall project 2 mm to left of the  $\frac{1}{2}\%$  graduations. Each 5% graduation shall be numbered (thus: 0, 5, 10, . . . 45, 50), the number being placed to left of scale. The capacity of neck

for each whole per cent on scale shall be 0.1 ml. The maximum error in total graduation or any part thereof shall not exceed volume of smallest unit of the graduation.

(2) *50%, 9-g, long-necked, 9" cream-test bottle.*—The same specifications shall apply to this bottle as to the 50%, 9-g, 6" cream-test bottle, except that total height of this bottle shall be 210–229 mm (8.25–9.0") and graduated portion of neck shall have a length of not less than 120 mm.

(3) *50%, 18-g, long-necked, 9" cream-test bottle.*—The same specifications shall apply to this bottle as to the 50%, 9-g, 9" cream-test bottle, except that charge of cream for this bottle shall be 18 g.

Each bottle shall bear on top of neck above graduations, in plain legible characters, a mark denoting weight of charge to be used, *viz.*, "9 g" or "18 g," as the case may be.

Each bottle shall be so constructed as to withstand the stress to which it will be subjected in centrifuge.

(4) *Testing.*—Proceed as directed under 21(a).

(b) *Water bath for cream samples.*—Provided with thermometer and device for maintaining temp. of 38–50°.

(c) *Cream weighing scales.*—With a sensibility reciprocal of 30 mg, *i.e.*, the addition of 30 mg to either pan of scale, when loaded to capacity, shall cause a deflection of at least 1 subdivision of graduation. The scales shall be set level upon a table support and be protected from drafts.

(d) *Weights.*—9 g and 18 g, respectively, and plainly marked "9 g" or "18 g," as the case may be. They shall be made of material capable of resisting corrosion or other injury, shall preferably be of a low squat shape, with rounded edges, and shall be verified at frequent intervals by comparison with standardized weights.

(e) *Acid measure.*—Described under 21(c).

(f) *Centrifuge or "tester."*—Described under 21(d).

(g) *Dividers or calipers.*—Described under 21(e).

(h) *Water bath for test bottles.*—Described under 21(f).

## 62

## DETERMINATION

Weigh 9 g of prepared sample, 54, directly into 9-g cream-test bottle, or 18 g into 18-g bottle, and proceed by one of following methods:

*Method 1.*—After cream has been weighed into test bottle, add 8–12 ml of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.82–1.83 at 20°) in the case of the 9-g bottle, or 14–17 ml in the case of the 18-g bottle, or add acid until mixture of cream and acid, after shaking, has assumed a chocolate-brown color. Shake until all lumps have completely disappeared and add 5–10 ml of soft  $\text{H}_2\text{O}$  at 60° or above. Transfer bottle to centrifuge, counterbalance it, and after proper speed has been attained whirl 5 min. Add soft hot  $\text{H}_2\text{O}$  until liquid column approaches top graduation of scale; then whirl 1 min. longer at temp. of 55–60°. Adjust temp. as directed under 22, and with aid of dividers or calipers measure fat column, in terms of percentage by weight, from its lower surface to bottom of upper meniscus.

*Method 2.*—*For 9-g bottle only.*—After cream has been weighed into test bottle, add 9 ml of soft  $\text{H}_2\text{O}$  and thoroly mix; add 17.5 ml of the  $\text{H}_2\text{SO}_4$  and shake until all lumps have completely disappeared. Transfer bottle to centrifuge, counterbalance it, and after the proper speed has been attained whirl 5 min. Fill bottle to neck with hot  $\text{H}_2\text{O}$  and whirl 2 min. Add hot  $\text{H}_2\text{O}$  until liquid column approaches top graduation of scale; and whirl 1 min. longer at temp. of 55–60°. Adjust temp. and measure fat column as directed under *Method 1*.

Whichever method is followed, the fat column, at time of reading, should be translucent, of golden yellow to amber color, and free from visible suspended parti-

cles. All tests in which the fat column is milky or shows presence of curd or of charred matter, or in which reading is indistinct or uncertain, should be rejected.

If glymol or pure white mineral oil (sp. gr. not to exceed 0.85 at 20°) is used, introduce a few drops only into bottle just before reading is made (it must not be dropped in, but must be allowed to flow down side of neck). For purpose of measurement, the surface separating the glymol and fat is regarded as representing the upper limit of the column. Oil-soluble artificial color may be added to the white mineral oil.

63 GELATIN—OFFICIAL.—See 29. Observe note.

64 PRESERVATIVES—OFFICIAL.—See 30 and XXXII.

65 COLORING MATTERS—OFFICIAL.—See 31 and XXI.

### EVAPORATED MILK (UNSWEETENED)

66 PREPARATION OF SAMPLE—OFFICIAL

(a) Transfer entire contents of can to large dish, stir thoroly, and pass thru fine sieve or strainer until homogeneous mass is secured. If slight separation of fat is evident, warm portion of sample containing separated fat to 30–35° and agitate until uniform emulsion is obtained; then combine with unheated portion and mix thoroly. (If an appreciable quantity of fat has separated, rendering impossible formation of satisfactory emulsion, an accurate analysis cannot be made.)

(b) Dilute 40 g of prepared homogeneous mass (a) with 60 g of H<sub>2</sub>O and mix thoroly.

67 TOTAL SOLIDS—OFFICIAL

Weigh 4–5 g of diluted sample, 66(b), into weighed flat-bottomed Pt dish, not less than 5 cm in diameter, and proceed as directed under 8. Correct result for the dilution.

68 ASH—OFFICIAL

Ignite residue from total solids determination, 67, at dull red heat until ash is free from C. Correct result for the dilution.

69 FAT—OFFICIAL

Weigh 4–5 g of undiluted sample, 66(a), into Röhrig tube or similar apparatus; dilute with H<sub>2</sub>O to ca 10.5 ml; and proceed as directed under 20, beginning "add 1.25 ml of NH<sub>4</sub>OH.

70 TOTAL NITROGEN—OFFICIAL

Weigh 5 g of undiluted sample, 66(a), transfer to Kjeldahl flask, and proceed as directed under II, 21, 22, or 23. Percentage of N  $\times 6.38$  = percentage of "proteins."

71 CASEIN—OFFICIAL

Weigh 10 g of diluted sample, 66(b), into beaker, and proceed as directed under 12 or 13. Correct result for the dilution.

72 ALBUMIN—OFFICIAL

Proceed as directed under 15, using filtrate from 71. Correct result for the dilution.

73

## LACTOSE—OFFICIAL

Proceed as directed under 17 or 19, using diluted sample, 66(b), and correct result for the dilution.

74

## GELATIN—OFFICIAL.—See 29.

75

## PRESERVATIVES—OFFICIAL.—See 30 and XXXII.

76

## COLORING MATTERS—OFFICIAL. See 31 and XXI.

## SWEETENED CONDENSED MILK

77

## PREPARATION OF SAMPLE—OFFICIAL

(a) If the can is cold, place it in H<sub>2</sub>O at 30–35° until warm. Open, scrape out all milk adhering to interior of can, and after transferring to dish sufficiently large to permit stirring thoroly mix until whole mass is homogeneous.

(b) Weigh 100 g of thoroly mixed sample into 500 ml flask, dilute to mark with H<sub>2</sub>O, and mix thoroly. If sample will not emulsify uniformly, weigh out separate portion of (a) for each determination.

78

## TOTAL SOLIDS—OFFICIAL

Use 10 ml of prepared soln, 77(b), and proceed as directed under 8, drying on either sand or asbestos fiber. Correct result for the dilution.

79

## ASH—OFFICIAL

Evaporate 10 ml of prepared soln, 77(b), to dryness on water bath and ignite residue as directed under XXXIV, 9 or 10. Correct result for the dilution.

80

## PROTEIN—OFFICIAL

Determine N as directed under II, 21, 22, or 23, using 10 ml of prepared soln, 77(b), and correct result for the dilution. Percentage  $N \times 6.38$  = percentage total nitrogen, "protein."

81

## LACTOSE—OFFICIAL

Dilute 100 ml of prepared soln, 77(b), in 250 ml volumetric flask to ca 200 ml; add 6 ml of Fehling's CuSO<sub>4</sub> soln, XXXIV, 32(a), and alkali soln of concentration and in proportion as directed in 19. Make up to mark, and mix thoroly. Filter thru dry filter and determine lactose as directed under XXXIV, 38. Correct result for the dilution.

82

## FAT—OFFICIAL

Weigh accurately 4–5 g of prepared sample, 77(a), into Röhrig tube or similar apparatus; dilute with H<sub>2</sub>O to ca 10.5 ml, and proceed as directed under 20, beginning "add 1.25 ml of NH<sub>4</sub>OH."

SUCROSE<sup>17</sup>—OFFICIAL

83

## REAGENT

*Mercuric nitrate soln.*—To 220 g of yellow HgO, add 300–400 ml of H<sub>2</sub>O and sufficient HNO<sub>3</sub> to form a clear soln (ca 140 ml), being careful to use least possible excess of acid. Dilute to 800–900 ml and add 10% NaOH slowly and with constant shaking until a slight permanent precipitate is obtained. Dilute to 1 liter and filter. As the soln tends to become acid with age owing to deposition of basic mercuric salts, dilute alkali should be added occasionally until a slight permanent precipitate is formed and the soln filtered.

84

## DETERMINATION

Introduce 50 ml of prepared soln, 77(b), into 100 ml flask; add 25 ml of H<sub>2</sub>O, mix, add 5 ml of the Hg(NO<sub>3</sub>)<sub>2</sub> soln, and shake thoroly. Without delay and while shaking constantly, add sufficient 0.5 N NaOH to render mixture neutral to litmus paper, being careful to avoid an alkaline reaction (usually 12-13 ml will be required). Dilute to 100 ml with H<sub>2</sub>O, mix thoroly, and filter thru dry paper. Polarize filtrate in 200 mm tube, then invert at room temp. as directed under XXXIV, 24(c), and polarize the inverted soln. Correct both readings for volume occupied by the protein, 80, and the fat, 82, 1 g of protein occupying a space of 0.8 ml and 1 g of fat, 1.075 ml. Calculate percentage of sucrose by following formula, using corrected direct and invert readings obtained above:

$$S = \frac{100(a-b)}{142.35 - \frac{t}{2}} \times \frac{26}{W}, \text{ in which}$$

$S$  = percentage of sucrose in the sample,

$a$  = corrected direct polarization,

$b$  = corrected invert polarization,

$t$  = temp. of soln polarized; and

$W$  = weight of sample taken (10 g).

## DRIED MILK AND MALTED MILK

85

SAMPLING DRIED MILK<sup>18</sup>—TENTATIVE

Use care to minimize any moisture absorption from air during sampling and avoid sampling on rainy day, or when humidity is high.

On surface of the milk at top of the barrel locate a point on each end of a diameter and on a radius perpendicular to this diameter, 1-2" in from edge of barrel. Midway on each side of triangle between these points locate a point. At the six points so located, using tubular trier sufficiently long to extend full length of barrel, draw a core parallel to vertical axis of barrel. Transfer cores to clean, dry, air-tight container and seal immediately.

Before opening sample for analysis, make homogeneous either by shaking or by alternately rolling and inverting container. Also avoid excessive temp. and humidity when opening sample container.

86

## PREPARATION OF SAMPLE—TENTATIVE

Sift sample thru a 20-mesh sieve onto large sheet of paper, rubbing material thru sieve and tapping vigorously if necessary. Grind residue in mortar, pass thru sieve, and mix into sifted material. Discard particles of wood and other material that cannot be ground. Sift sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve sample in air-tight container.

87

MOISTURE<sup>19</sup>—TENTATIVE

Weigh 1-1.5 g of sample into a round flat-bottomed metal dish (not less than 5 cm in diameter and provided with close-fitting slip-in cover). Loosen cover and place dish on metal shelf (dish resting directly on shelf) in vacuum oven kept at temp of boiling H<sub>2</sub>O. Dry to constant weight (ca 5 hours) under pressure not to exceed 100 mm (4") of Hg. During drying admit into oven a slow current of air (ca 2 bubbles per second), dried by passing thru H<sub>2</sub>SO<sub>4</sub>. Discontinue action of vacuum pump and

carefully admit dried air into oven. Press cover tightly into dish, remove from oven, cool, and weigh. Calculate percentage loss in weight as moisture.

88

## PROTEIN—TENTATIVE

Weigh 1 g of sample into Kjeldahl digestion flask and determine N as directed under II, 23. Percentage of  $N \times 6.38$  = percentage of "protein."

89

## ASH—TENTATIVE

Ignite 1 g of sample at dull red heat until free from C. If suitable dish was used for moisture determination, 87, ash may be determined on portion there used. Cool in desiccator and weigh.

90

FAT IN MALTED MILK<sup>20</sup>—TENTATIVE

Weigh quickly ca 1 g of well-mixed sample into small, lipped beaker. Add 1 ml of  $H_2O$  and rub up to smooth paste. Add 10 ml more of  $H_2O$ , warm on steam bath, and transfer to Röhrig tube or similar apparatus with the aid of 10 ml of alcohol. Mix thoroly, cool, and proceed as directed under 20, beginning "add 25 ml of ether," rinsing beaker with this ether.

FAT IN DRIED MILK<sup>21</sup>—OFFICIAL

91

## PREPARATION OF SOLUTION

Proceed as directed in one of following methods:

(a) Weigh quickly ca 1 g of well-mixed sample into small, lipped beaker. Add 1 ml of  $H_2O$  and rub up to smooth paste. Add 9 ml more of  $H_2O$  and 1 ml of  $NH_4OH$  and warm on steam bath. Transfer to Röhrig tube or similar apparatus. Cool, and proceed as directed in 92, rinsing beaker successively with the alcohol and ethers used in first extraction.

(b) Weigh quickly ca 1 g of well-mixed sample and transfer to Röhrig tube or similar apparatus. Add 10 ml of  $H_2O$  and shake until homogeneous, warming if necessary. Add 1 ml of  $NH_4OH$  and heat in water bath at 60–70° for 15 min., shaking occasionally. Cool, and proceed as directed in 92.

92

## DETERMINATION

Add 10 ml of alcohol, and mix. Extract with ethyl ether and petroleum benzin as directed under 20. For the second extraction add 4 ml of alcohol, and again extract as directed under 20. With whole milk and cream powders make a third extraction, using 15 ml of each solvent after adding, if necessary, sufficient  $H_2O$  to bring aqueous layer in tube to its original volume.

93

## CITRIC ACID IN DRIED MILK—TENTATIVE

Weigh 6 g of well-mixed sample, mix well with 44 ml of  $H_2O$ , and proceed as directed under 5, beginning "add ca 100 mg of tartaric acid."

94

MICROSCOPICAL IDENTIFICATION OF MALTED MILK AND ITS FLAVORED PRODUCTS<sup>22</sup>—TENTATIVE

Mount small quantity of material in drop of mineral oil on slide, apply cover-glass, and examine preparation at a magnification of ca 200, using microscope lamp with daylight glass as a source of light. Control light intensity by the iris diaphragm because a too brilliantly lighted field hinders recognition of details. (See Figs. 29 and 30.)

## BUTTER

(Methods are also applicable to renovated or process butter and margarine.)

95

SAMPLING<sup>24</sup>—TENTATIVE

(a) *Tub or cube butter*.—Insert regular trough butter trier practically its full length from a point near top edge (or corner in case of cube) thru center to point at bottom diagonally opposite point of entry. Give trier one complete turn and withdraw full core. Hold point of trier over mouth of sample container and immediately transfer core of butter in ca 3" sections, working it from trier by aid of spatula fitted to groove. Leave plug ca 1" long to place in hole from which core was removed. Add two other trierfuls taken similarly at points equidistant with the first (two other corners in case of cube) to the jar to constitute subdivision from tub or cube sampled. Do not include moisture adhering to outside of trier. Clean and dry trier before each drawing. Use an unwarmed trier for butter stored above freezing point. For harder butter use trier warmed to temp. that may be just borne by hand. Soften butter frozen so hard as to resist trier by storage in tempering room for 24 hours.

Sample lots as follows:

(1) *Tubs (or cubes) marked with churn numbers*.—Sample one tub of each churn of 1-9 tubs, two of each churn of 10-14 tubs, and three of each churn of over 14 tubs. In no case sample less than two tubs in a lot.

(2) *Tubs not marked with churn numbers*.—Sample a number of tubs equivalent to square root of number in lot, with a minimum of 3 and maximum of 25. If square root is not a whole number, sample one extra tub.

(b) *Print butter*.—Withdraw one print from each of a number of cases equivalent to square root of number of cases in the lot, with minimum of 5 and maximum of 25. When the square root is not a whole number, sample one extra case. Select cases to include each churn or batch mark when so marked. With less than 5 cases sample all, taking 5 prints as a minimum. Remove wrapper and transfer each print to separate sample container. (With prints of 1 pound or over the print may be quartered and two opposite quarters selected as sample.) With 8 oz and 4 oz prints, take whole print as sample.

These directions provide minimum sampling, to be increased if object of examination demands.

(c) *Sample containers*.—Use a glass jar, preferably with glass top, of such type as will prevent loss of moisture by evaporation or entrance of H<sub>2</sub>O into jar. Tops containing a liner of any material should not be used.

96

PREPARATION OF SAMPLE<sup>24</sup>—OFFICIAL

Soften entire sample in closed vessel by heating in water bath kept at 39°, and shaking occasionally until temp. of entire mass is 35°; or by heating in constant temp. oven at 35° until temp. of entire mass has reached 35°. Shake vigorously until a homogeneous semi-solid mass is obtained. Weigh the portion for analysis at once. If sample is kept for any length of time, again soften and prepare as directed above before withdrawing portions for analysis.

## MECHANICAL STIRRER METHOD—TENTATIVE

97

## EQUIPMENT

(a) *Stirrer*.—Electric food mixer of double beater type with variable speed control and geared down to give maximum speed of 1000 r.p.m.; beater chucks should be on 1½" centers, and motor housing be fitted with clamp for use on ring stand.



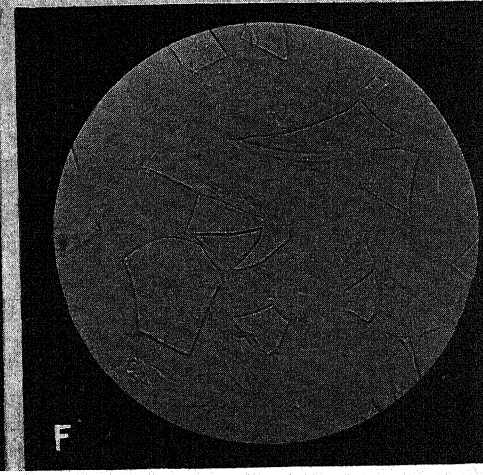
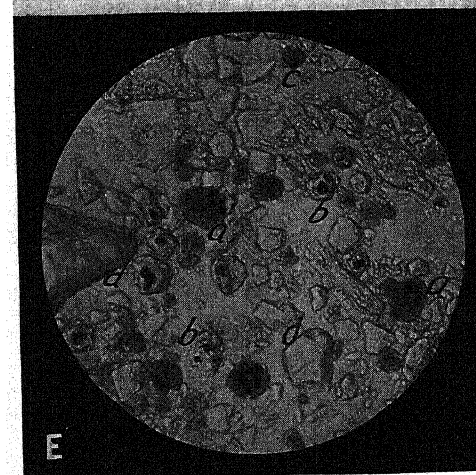
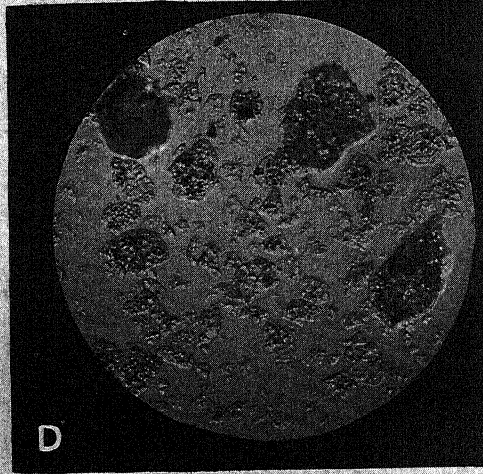
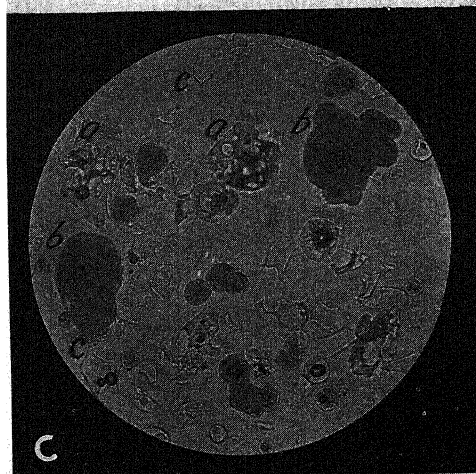
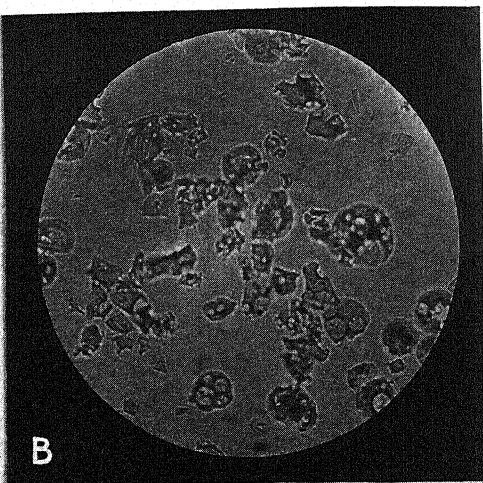
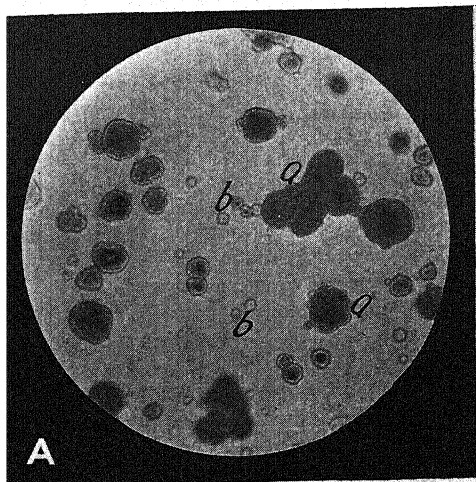


FIG. 29.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

## DESCRIPTION OF THE PHOTOMICROGRAPHS

## FIGURE 29

A.—SPRAY-DRIED WHOLE MILK; (a) MILK MASSES, (b) FAT GLOBULES; B.—SPRAY-DRIED MALT EXTRACT; C.—MECHANICAL MIXTURE; (a) SPRAY-DRIED MALT EXTRACT, (b) MILK MASSES, (c) FAT GLOBULES; D.—SPRAY-DRIED SKIM MILK; E.—MECHANICAL MIXTURE; (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; F.—DRUM-DRIED MALT EXTRACT.

A represents spray-dried whole milk. Large particles represent aggregates of globular milk masses having stippled surface (a). Fat appears as droplets (b).

B represents a spray-dried malt extract having appearance of aggregates of droplets enclosed in spherical masses.

C represents a product made by mixing spray-dried whole milk and the spray-dried malt extract, shown in A and B, in proportion necessary to give approximate composition of malted milk. The globular stippled milk masses (b) and the malt extract masses (a) are easily recognized; (c) fat globules.

D represents spray-dried skim milk that might be confused with the spray-dried malt extract (B) because structure of spherical masses is similar. A comparison of the 2 pictures, however, will show that the droplets in the malt extract masses are larger than those appearing in the milk masses.

E represents a product purchased on market and represented to contain malt, skim milk, whole milk, cocoa, and sugar. Examination shows malt extract (b), dried skim milk (a), cocoa (c), and sugar (d) present. Whole milk is absent. The cocoa consists of brown amorphous particles, easily discernible under the microscope. The highly refractive, irregular fragments of sugar cannot be mistaken.

F represents a drum-dried malt extract. It consists of clear, highly refractive fragments closely resembling broken glass.

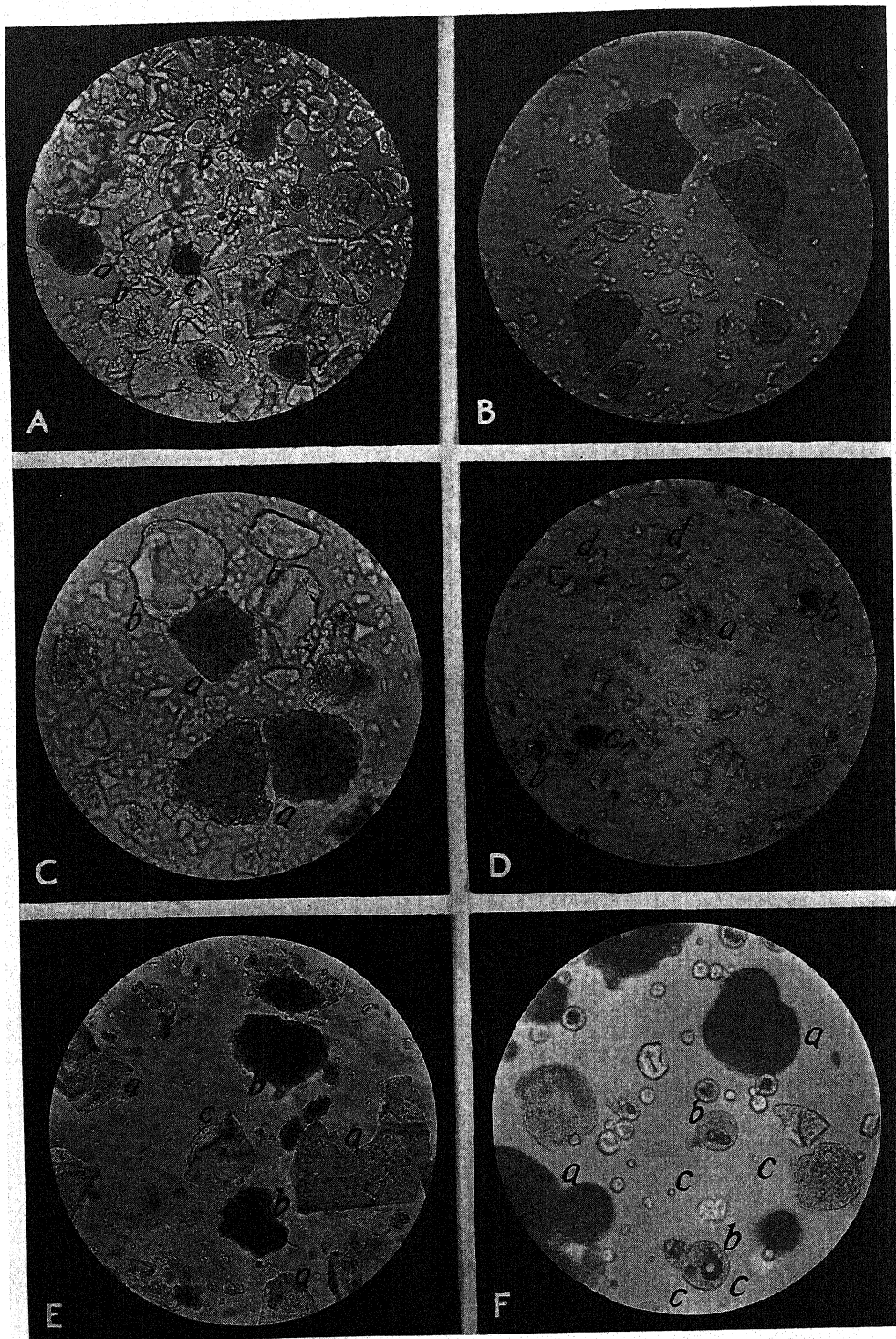


FIG. 30.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

## DESCRIPTION OF THE PHOTOMICROGRAPHS

## FIGURE 30

A.—MECHANICAL MIXTURE; (a) MILK MASSES, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; B.—MALTED MILK; C.—SWEET CHOCOLATE FLAVOR MALTED MILK; (a) MALTED MILK MASSES, (b) SUGAR; D.—MECHANICAL MIXTURE; (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; E.—MECHANICAL MIXTURE; (a) MALTED MILK MASSES, (b) COCOA, (c) SUGAR; F.—PRODUCT PREPARED FROM MALT INFUSION AND MILK BY SPRAY DRYING; (a) MILK MASSES, (b) MALT EXTRACT MASSES, (c) FAT GLOBULES.

A represents mechanical mixture of dried whole milk (A, Fig. 29), dried malt extract (B, Fig. 29), cocoa and sugar. The milk (a) and malt extract (b) masses and sugar particles (d) are readily recognizable. A mass of cocoa appears near center of picture (c).

B is characteristic of genuine malted milks of the market. This picture cannot be mistaken for any product of similar composition. The malt extract solids and the milk solids are incorporated into homogeneous irregular fragments having a stippled surface.

C represents a "sweet-chocolate-flavor malted milk" purchased on market, prepared by simultaneously evaporating in vacuo milk, malt infusion, cocoa and sugar. It is easy to recognize characteristic malted milk masses (a), shown in the picture immediately preceding. They are slightly thicker and appear darker in picture because the cocoa is intimately associated with them.

D represents a mechanical mixture of spray-dried skim milk (D, Fig. 29), spray-dried malt extract (B, Fig. 29), cocoa and sugar. No trouble is experienced in identifying these materials.

E represents a mechanical mixture of malted milk (B), cocoa, and sugar. Examination shows that malted milk (a) is present.

F represents a product found on market under label "malted milk." It shows none of characteristics found in genuine malted milk, as is readily seen by a comparison with B. Individual milk masses (a), fat globules (c), and malt extract masses (b) closely resembling the spray-dried products (A and B, respectively, Fig. 29) predominate. Some particles show stippled surface of genuine malted milk, but they are spherical instead of angular. A comparison of this picture with picture showing mechanical mixture of spray-dried whole milk and spray-dried malt extract (C, Fig. 29) shows striking similarity.

(b) *Paddles*.—Brass, of dimensions given in Fig. 31. Ends of shafts should be fitted to beater chucks so as to be easily removable; blades are made of 22 gage spring brass, soldered to slot in shaft.

(c) *Cover*.—Preferably made of light metal,  $4\frac{1}{2}$ " in diameter; holes for shafts should be slightly oversize to permit cover to move easily up or down while paddles are turning.

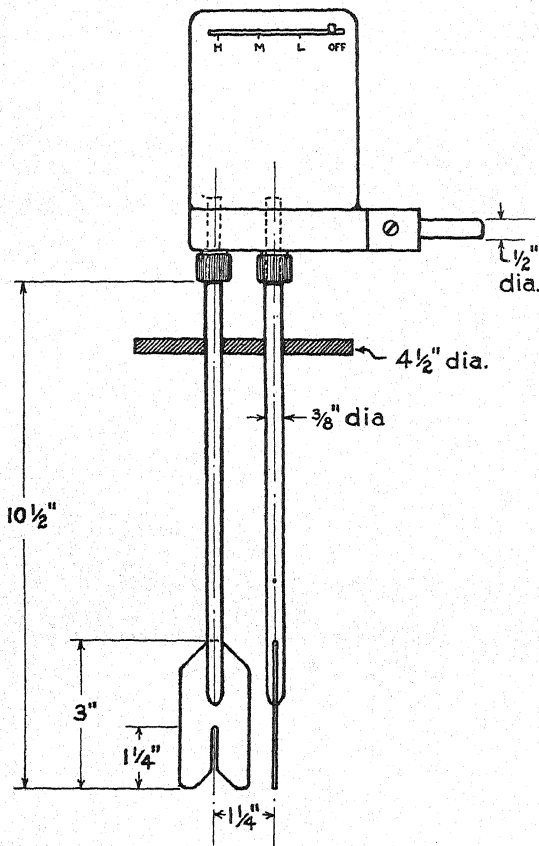


FIG. 31.—MECHANICAL STIRRER FOR PREPARATION OF BUTTER SAMPLES.

with 15 ml of absolute ether or petroleum benzin; transfer to weighed Gooch crucible with aid of wash bottle filled with the solvent; and wash free from fat with 100 ml of the solvent. (Last 25 ml of the 100 ml of solvent should pass thru the crucible without aid of suction.) Dry crucible and contents at temp. of boiling  $H_2O$  until weight is constant. Repeat the washing with 25 ml of solvent and dry to constant weight. Repeat operation until there is no loss in weight due to washing.

101

#### *Direct Method—Official*

From the dry butter, obtained in determination of moisture either with or with-

#### 98 PREPARATION

Warm entire sample in the sample jar, 95(c), until sufficiently softened to be readily stirred ( $25-30^\circ$ ). Insert paddles, with cover in place, and mix at high speed for 2-3 min. Raise, lower, and rotate jar until all portions of sample are incorporated in the mixture. Remove paddles and replace jar top. Remix sample if weighing portion for analysis is delayed over 3 hours or after temp. goes above  $30^\circ$  or below  $23^\circ$ .

#### 99 MOISTURE—OFFICIAL

Weigh 1.5-2.5 g of prepared sample, 96, into a flat-bottomed dish not less than 5 cm in diameter and dry to constant weight in oven kept at temp. of boiling  $H_2O$ . Clean, dry sand or asbestos may be used if fat is not to be determined in residue by 100.

#### FAT<sup>24</sup>

#### 100 *Indirect Method—Official*

Take up the dry butter, obtained in moisture determination in which no absorbent was used, 99, by macerating

out use of an absorbent, extract the fat with anhydrous, alcohol-free ether, or with petroleum benzin (b.p. below 65°), receiving the soln in weighed flask. Evaporate the solvent and dry extract to constant weight at temp. of boiling H<sub>2</sub>O.

102

## CASEIN, ASH, AND SALT—OFFICIAL

Cover crucible containing residue from fat determination by the indirect method, 100, and heat, gently at first. Raise temp. gradually to not over 500°, remove cover, and continue heating until contents of crucible are white. Loss in weight represents casein, and residue in the crucible, mineral matter. Dissolve this mineral matter in H<sub>2</sub>O slightly acidified with HNO<sub>3</sub> and determine Cl, either gravimetrically as directed under XII, 35, or volumetrically as directed under XII, 37, and calculate the NaCl.

103

## SALT—OFFICIAL

Weigh in counterpoised beaker 5–10 g of sample; add ca 20 ml of hot H<sub>2</sub>O; and after butter has melted transfer the whole to separatory funnel. Insert stopper and shake for a few minutes. Let stand until all fat has collected on top of the H<sub>2</sub>O; then draw off H<sub>2</sub>O into flask, being careful to let none of fat globules pass. Again add hot H<sub>2</sub>O, rinsing beaker, and repeat extraction 10–15 times, using 10–20 ml of H<sub>2</sub>O each time. The washings will contain all but mere trace of the NaCl originally present in the butter. Determine quantity in whole or in an aliquot of the liquid by titration with standard AgNO<sub>3</sub>, using K<sub>2</sub>CrO<sub>4</sub> indicator.

104

## EXAMINATION OF FAT—OFFICIAL

Melt the butter and keep in dry place at ca 60° for 2–3 hours, or until H<sub>2</sub>O and curd have entirely separated. Filter the clear, supernatant fat thru dry filter paper in hot water funnel or in an oven at ca 60°. If the filtered liquid fat is not perfectly clear, refilter. Determine physical and chemical constants as directed under XXXI.

105

## COLORING MATTERS—OFFICIAL

Pour ca 2 g of the filtered fat, dissolved in ether, into each of 2 test tubes. Into one of tubes pour 1–2 ml of HCl (1+1) and into other about same volume of 10% NaOH soln. Shake tubes well and allow to stand. In presence of azo dyes the test tube to which the acid has been added will show a pink to wine-red coloration, while the alkaline soln in the other tube will show no color. If, on the other hand, annatto or other vegetable color is present, the alkaline soln will be colored yellow, while no color will be apparent in the acid soln.

*General test.*—Proceed as directed under XXI, particularly 3 and 16(b), for the detection of oil-soluble coal tar dyes and annatto.

106

## PRESERVATIVES—OFFICIAL.—See 30 and XXXII.

107

## MICROSCOPIC EXAMINATION—OFFICIAL

(a) Place on slide a small portion of the fresh unmelted sample taken from the inside of the mass, add a drop of pure olive oil, apply cover-glass with gentle pressure, and examine with magnification of 120–150 diameters for crystals of lard, etc. Examine another portion of sample with polarized light and selenite plate without use of oil. Pure fresh butter will show neither crystals nor a parti-colored field with



selenite. Renovated butter or other fats melted and cooled and mixed with butter will usually present crystals and variegated colors with the selenite plate.

(b) For further microscopic study dissolve in test tube 3–4 ml of the fat in 15 ml of ether. Close tube with loose plug of cotton wool and allow to stand 12–24 hours at 20–25°. When crystals form at bottom of tube, remove with pipet, glass rod, or tube; place on slide, cover, and examine under microscope. The crystals formed by later deposits may be examined in similar way. Compare with crystals obtained in same manner from samples of known purity.

#### MOLD MYCELIA IN BUTTER<sup>26</sup>—TENTATIVE

108

##### REAGENT

*Gum soln.*—Make up 1 liter of 0.75% soln of carob bean gum with 2% of added formaldehyde as preservative. (The dry gum may be conveniently added by first mixing it in 10–15 ml of alcohol and stirring this mixture rapidly into the H<sub>2</sub>O.) Gently heat soln to boiling to drive off alcohol and air, and continue heating 25–30 min. Add formaldehyde on cooling. Use the clear supernatant soln, free from cells, left when cellular elements in gum gradually settle out. (A similar soln made with gum tragacanth may also be used for this purpose.)

109

##### PROCEDURE

Make a careful examination of surface of butter and note any mold growth visible. To remove possibility of contamination by any surface mold not visible, scrape off and discard  $\frac{1}{8}$ " of surface, after which take sample from the exposed surface.

Weigh out 1 g of butter by means of  $\frac{1}{2}$  teaspoon measure. Measure out 7 ml of the hot gum soln and, with spoon bottom-side-up over 50 ml beaker, pour 2 or 3 ml of the hot soln over spoon to loosen butter. Use remainder of hot soln to complete rinsing of fat from spoon. Stir until mixture is uniform and fat globules are 0.1–0.2 mm in diameter. (Stirring necessary must be determined by experience.)

Mount a portion of the mixture on the mold-counting slide and estimate mold as directed under XXXV, 30, 31. Report no field positive unless combined length of two longest filaments exceeds  $\frac{1}{2}$  of diameter of field. *Alternative staining procedure.*—Add 1 or 2 drops of 5% crystal violet soln to gum-butter mixture after butter is melted. Mix preparation thoroly and prepare slide as directed in original method.

#### RENOVATED BUTTER<sup>26</sup> AND OLEOMARGARINE

110

##### I. Foam Test—Tentative

Heat 2–3 g of sample in either a spoon or a dish over small flame. True butter will foam copiously, whereas process butter will bump and sputter like hot grease, with little or no foaming. Oleomargarine behaves like process butter, but chemical tests will determine whether sample is oleomargarine or butter.

111

##### II. Melted Fat Test—Tentative

Melt 50–100 g of butter or renovated butter at 50°. The curd from butter will settle, leaving clear supernatant fat; in the case of renovated butter, the supernatant fat remains more or less turbid.

#### CHEESE

112

##### SELECTION AND PREPARATION OF SAMPLE—OFFICIAL

When the cheese can be cut, take a narrow, wedge-shaped segment reaching from the outer edge to the center. Cut this segment into strips and pass 3 times thru

sausage machine. When the cheese cannot be cut, take sample with cheese trier. If only one plug can be obtained, take it perpendicularly to surface of the cheese at point  $\frac{1}{3}$  distance from edge to center and extending either entirely or half way thru it. When possible draw 3 plugs, 1 from the center, 1 from a point near the outer edge, and 1 from a point half-way between the other two. For inspection purposes reject rind, but for investigations requiring absolute quantity of fat in cheese include rind in sample. Grind the plugs in sausage machine (preferable method), or cut them very finely and mix thoroly.

113

MOISTURE<sup>27</sup>—OFFICIAL

Weigh 2–3 g of prepared sample, 112, into round flat-bottomed metal dish, not less than 5 cm in diameter and provided with close-fitting slip-in cover. In the case of soft cheese and process cheese of high moisture content, weigh 1–2 g and partially dry on steam bath. Place the loosely covered dish on metal shelf (dish resting directly on shelf) in vacuum oven, kept at temp. of boiling H<sub>2</sub>O. Dry to constant weight (ca 4 hours) under pressure not to exceed 100 mm (4") of Hg. During drying admit into oven slow current of air (ca 2 bubbles per second) dried by passing thru H<sub>2</sub>SO<sub>4</sub>. Discontinue action of vacuum pump and carefully re-admit air into oven. Press cover tightly into dish, remove dish from oven, cool, and weigh. Express the loss in weight as moisture.

114

ASH<sup>28</sup>—OFFICIAL

Weigh into a Pt dish 3–5 g of prepared sample, 112, place on steam bath, and dry ca 1 hour. (If cheese is rich in fat, a small amount of absorbent cotton may be placed in dish.) Ignite cautiously to avoid spattering and remove burner while fat is burning. When flame has died out, complete ignition in muffle at temp. not exceeding 550°.

115

TOTAL CHLORIDES<sup>29</sup>—OFFICIAL

Weigh ca 3 g of prepared sample, 112, into a 300 ml Erlenmeyer flask and add 25 ml of 0.1 N AgNO<sub>3</sub>, which is more than enough to combine with all the Cl. Add 10 ml of halogen-free HNO<sub>3</sub> and 50 ml of H<sub>2</sub>O and boil. As soln boils add ca 15 ml of 5% KMnO<sub>4</sub> soln in 5 ml portions. (Soln becomes yellowish and clear.) Cool; filter the soln into a 200 ml graduated flask, washing the filter paper thoroly with H<sub>2</sub>O at ca 20°; and make to volume. Titrate excess AgNO<sub>3</sub> in 100 ml of the clear soln with 0.1 N KSCN, using 2 ml of saturated soln of Fe alum as indicator. Run blank on reagents used, following same procedure, except to add sugar to destroy excess of permanganate. Calculate Cl found to NaCl.

116

## NITROGEN—OFFICIAL

Determine N in a weighed portion (ca 2 g) of prepared sample, 112, as directed under II, 21, 22, or 23. Percentage of N  $\times 6.38$  = percentage of N, "protein."

117

## ACIDITY—OFFICIAL

To 10 g of prepared sample, 112, add H<sub>2</sub>O at 40° until volume equals 105 ml, shake vigorously, and filter. Titrate 25 ml portions of the filtrate, representing 2.5 g of the sample, with standard NaOH, preferably 0.1 N, using phenolphthalein. Express result in terms of lactic acid. 1 ml of 0.1 N NaOH = 0.0090 g of lactic acid.

118

## COLORING MATTERS—TENTATIVE

Extract 25–50 g of prepared sample, 112, with ether, remove ether by evaporation and proceed as directed under XXI, 3 or 15.



In a small, narrow (or tall form), beaker rub to a smooth liquid 1 g of prepared sample, 112, with 9 ml of  $H_2O$  and 1 ml of  $NH_4OH$ . Digest mixture at low heat until casein is well softened; neutralize with  $HCl$ , using litmus as indicator; and add 10 ml more of  $HCl$ . Add ca 0.5 g of sand previously digested with  $HCl$  to prevent bumping and boil gently for 5 min., keeping beaker covered with watch-glass. Cool soln, transfer to Röhrig tube or similar apparatus, rinse beaker with 25 ml of ether, and transfer ether rinsings to Röhrig tube, shaking thoroly. Add 25 ml of petroleum benzin (b.p. below  $65^\circ$ ), shake thoroly, and let mixture separate. Proceed as directed under 20, beginning "Draw off into a suitable flask or metal dish thru small, quick-acting filter as much as possible of the ether-fat soln."

(a) *Alkaline extraction*.—In a large, wide-necked flask, treat ca 300 g of the cheese, cut into fragments the size of a pea, with 700 ml of 5%  $KOH$  soln at  $20^\circ$ , shaking vigorously to dissolve the casein. In 5–10 min. the casein will be dissolved, and the fat will rise to surface in lumps. Collect the lumps of fat into as large a mass as possible by shaking gently. Pour cold  $H_2O$  into flask until the fat is driven up into the neck and remove it by suitable means. Wash fat thus obtained with just sufficient  $H_2O$  to remove residue of alkali that it may contain. The fat is not perceptibly attacked by the alkali in this treatment, is practically all separated in short time, and is then easily prepared for chemical analysis by filtering and drying as directed under 104. Examine fat as directed under XXXI.

(b) *Acid extraction*.—Pass the cheese thru a grinding machine, transfer to large flask, and cover with warm  $H_2O$ , using 1 ml for every gram of cheese. Shake thoroly and add  $H_2SO_4$  slowly and in small quantities, shaking after each addition of acid. (Volume of  $H_2SO_4$  should equal volume of  $H_2O$  used.) Remove fat, which separates after standing a few minutes, by means of a separatory funnel; wash free from sulfate, filter, and dry as directed under 104. Examine fat as directed under XXXI.

#### TARTARIC ACID<sup>30</sup>

##### *Qualitative Test—Tentative*

To 5 g of the ground cheese, 112, add 40 ml of  $H_2O$  at temp. of ca  $50^\circ$  and shake until cheese is thoroly broken up. Add 3 ml of 1%  $H_2SO_4$  soln and shake vigorously. Add 2 ml of 20% soln of phosphotungstic acid and again shake vigorously. Let stand 5 min. and filter. To 25 ml of filtrate add sufficient saturated  $Ba(OH)_2$  soln to make alkaline and 25 ml of alcohol, shake vigorously, and allow to settle. Filter thru Büchner funnel, using light suction, and wash residue on filter several times with  $H_2O$ . Transfer portion of the paste to small evaporating dish and dry on steam bath. Add a few ml of  $H_2SO_4$  and a few crystals of resorcin, and heat slowly. If tartaric acid is present, there is produced a rose-red color that is slowly discharged on dilution with  $H_2O$ .

##### *Quantitative Method<sup>31</sup>—Official*

(a) *Potassium chloride wash soln*.—Dissolve 15 g of  $KCl$  in 100 ml of  $H_2O$  and add 20 ml of alcohol.

(b) *Tartaric acid soln*.—Dissolve 1.5 g of pure tartaric acid in previously boiled and cooled  $H_2O$  and dilute to 100 ml at  $20^\circ$ . Titrate with 0.1  $N$   $NaOH$  to determine quantity of tartaric acid in 10 ml of the soln.

123

## DETERMINATION

Weigh 25 g of prepared sample, 112, into 500 ml salt-mouth bottle and add, 25 ml at a time, 100 ml of  $H_2O$  at  $50-60^\circ$ , shaking vigorously after each addition. If necessary, continue shaking until cheese is thoroly broken up. Add 25 ml of 2% Na oxalate soln and shake vigorously 1 min. Add 100 ml of 2% HCl, 25 ml at a time, shaking vigorously after each addition. Add 50 g of powdered KCl, and shake 5 min. To avoid churning, keep mixture warm (ca  $50^\circ$ ) during shaking. Transfer contents of bottle, with aid of  $H_2O$ , to 300 ml flask, cool to  $20^\circ$ , and make up to mark with  $H_2O$ . Mix thoroly; let stand 10 min., with occasional shaking, and filter thru dry folded filter, discarding first few ml of filtrate. Disregard any opalescence and transfer 200 ml of filtrate to 250 ml flask. Neutralize with 1 *N* NaOH, using phenolphthalein, and add 5.2 ml in excess. Make up to mark with  $H_2O$ , mix thoroly, let stand a few minutes, and filter thru dry folded filter, discarding first few ml of filtrate. To 100 ml of filtrate in a 250 ml beaker add, with constant stirring, 10 ml of the tartaric acid soln, 2 ml of glacial acetic acid, and 23 ml of alcohol. Cool in ice bath, stir vigorously until the cream of tartar begins to crystallize, and let stand in refrigerator overnight. Prepare a Gooch crucible having a removable disk with pad of asbestos ca 10 mm thick. Decant most of liquid thru this filter, wash precipitate into crucible with the KCl wash soln, and wash the beaker and precipitate 3 times, using a total quantity of 20-30 ml of the wash soln. Place asbestos and precipitate in the beaker in which precipitation was made and wash crucible with ca 50 ml of hot  $H_2O$ . Heat soln to boiling and titrate while hot with 0.1 *N* NaOH, using phenolphthalein. Calculate percentage of tartaric acid in cheese by means of formula:

$$X = 14.26[0.015(B + 1.5) - A], \text{ in which}$$

$A$  = g of tartaric acid in 10 ml of the tartaric acid reagent; and

$B$  = ml of 0.1 *N* NaOH required for titration.

In the factor 14.26 the concentration caused by the insoluble solids of cheese of average composition is also taken into consideration.

CITRIC ACID<sup>22</sup>

124

*Qualitative Test—Tentative*

To 10 g of prepared sample, 112, add 20 ml of  $H_2O$  at ca  $50^\circ$  and shake vigorously until cheese is thoroly broken up. Add 20 ml of  $H_2SO_4$  (1 + 1) and 2 ml of 20% soln of phosphotungstic acid, and shake vigorously. Let stand for 5 min. and filter. To 20 ml of filtrate add 10 ml of Br water and 5 ml of KBr soln (15 g in 40 ml of  $H_2O$ ) and proceed with oxidation as directed in the following quantitative determination. Add sufficient  $FeSO_4$  soln to dissolve precipitated  $MnO_2$ . If citric acid is present, a heavy white precipitate that settles rapidly is formed.

125

*Quantitative Method<sup>23</sup>—Official*

Weigh 25 g of prepared sample, 112, into a 500 ml salt-mouth bottle, and add, 25 ml at a time, 100 ml of  $H_2O$  at  $50-60^\circ$ , shaking vigorously after each addition. Continue shaking until cheese is thoroly broken up. Add 25 ml of 2% Na oxalate and shake vigorously 1 min. Add 100 ml of 1%  $H_2SO_4$ , 25 ml at a time, shaking vigorously after each addition. Add 3 ml of 20% soln of phosphotungstic acid and shake; then add 25 g of powdered anhydrous  $Na_2SO_4$ , and shake 5 min. To avoid churning, keep mixture warm (ca  $50^\circ$ ) during shaking. Transfer contents of bottle with aid of warm  $H_2O$  to 300 ml volumetric flask, cool to  $20^\circ$ , and make up to mark with  $H_2O$ . Mix thoroly, shake occasionally during 10 min., then filter thru dry folded filter, discarding first few ml of filtrate. Heat 200 ml of filtrate to boiling and while

still hot add 20 ml of  $\text{H}_2\text{SO}_4$  (1+1) and 2 ml of the phosphotungstic acid. Mix and allow to stand 15 min. With aid of  $\text{H}_2\text{O}$  transfer mixture to 250 ml flask, cool to  $20^\circ$ , make up to mark with  $\text{H}_2\text{O}$ , and filter thru dry folded filter. Transfer 100 ml of clear filtrate to 500 ml Erlenmeyer flask (ca 0.3 g of washed and dried asbestos may be added). Add 10 ml of freshly prepared saturated Br water and 5 ml of KBr soln (5 g KBr in 40 ml of  $\text{H}_2\text{O}$ ), mix thoroly, and heat to  $48-50^\circ$ . Hold at this temp. for 5 min., add 25 ml of 5% permanganate, shake, and allow to stand ca 5 min. Cool flask and contents to ca  $8^\circ$ , add 40 ml of cold  $\text{FeSO}_4$  soln (20 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$  and 1 ml of  $\text{H}_2\text{SO}_4$ ), shake continuously 5 min., and let mixture stand overnight in refrigerator. Decant supernatant liquid thru Gooch crucible, measure volume of filtrate (a) and wash precipitate from the Erlenmeyer into crucible with this filtrate. Wash precipitate with 3 successive 20 ml portions of ice-cold  $\text{H}_2\text{SO}_4$  (1+100), sucking dry after each addition, and finally wash with 3 successive 20 ml portions of ice-cold  $\text{H}_2\text{O}$ . Dry precipitate to constant weight over  $\text{H}_2\text{SO}_4$  in vacuum desiccator, protecting precipitate from strong light or, to save time, dry in current of air passed thru  $\text{H}_2\text{SO}_4$ . Weigh, and remove pentabromacetone by extracting first with 3 successive 20 ml portions of alcohol and then with 3 successive 20 ml portions of ether. Dry, and weigh crucible. To weight of pentabromacetone add 0.004 g for each 100 ml of filtrate (a) to compensate for solubility of pentabromacetone and multiply result by 6.06 to obtain percentage of anhydrous citric acid in the cheese. (In this factor consideration is taken of the concentration caused by the insoluble solids in 25 g of cheese. It is assumed that the solids of cheese are almost insoluble under the conditions maintained and that average process cheese contains ca 60% of solids. No allowance is made for variation in the salt or moisture content or for variation in the specific volume of the solids, as such variations do not appreciably affect results.)

#### LACTOSE IN PROCESS CHEESE<sup>24</sup>

126

##### *Quantitative Method*

Weigh 25 g of prepared sample, 112, into 500 ml salt-mouth bottle, and add in 25 ml portions 100 ml of  $\text{H}_2\text{O}$  at  $50-60^\circ$ , shaking vigorously after each addition. Continue shaking until cheese is thoroly broken up. Add 25 ml of 2% Na oxalate and shake vigorously 1 min.; add 25 g of powdered  $\text{Na}_2\text{SO}_4$  and shake 2 min.; add 10 ml of  $\text{H}_2\text{SO}_4$  (1+1) and shake; then add 25 ml of 20% phosphotungstic acid and shake vigorously. Transfer contents of bottle to 500 ml flask, cool at once to  $20^\circ$ , and make to mark with  $\text{H}_2\text{O}$ . Mix thoroly, allow to stand 10 min., and filter thru dry folded filter. Transfer 150 ml of filtrate to each of two 250 ml flasks, add 10% NaOH to one flask until mixture is alkaline to litmus, then add 5 g of solid KCl and mix thoroly. Cool to  $20^\circ$  and make to mark with  $\text{H}_2\text{O}$ . Shake well, allow to stand 10 min., and filter thru dry folded filter. Determine lactose in 50 ml aliquot as directed in XXXIV, 38. Treat 50 ml of the 150 ml in the second flask as directed in XXXIV, 24(c), using 10 ml of HCl, etc. Add 10% NaOH until alkaline to litmus, and add 5 g of solid KCl. Mix thoroly, cool to  $20^\circ$ , and make to mark with  $\text{H}_2\text{O}$ . Let stand 10 min. Filter if necessary thru dry filter paper. Determine lactose in 50 ml aliquot as before. An agreement between amount of  $\text{Cu}_2\text{O}$  reduced before and after inversion establishes absence of sucrose.

Since the insoluble material of cheese and the precipitated phosphotungstic acid occupy some space in the flask as originally made up, it is necessary to correct for this volume. From the average composition of cheese the volume of the precipitate was calculated to be 14 ml. To obtain the true amount of lactose present, multiply all results by the factor 0.97.

GUMS IN SOFT CURD CHEESE<sup>33</sup>—TENTATIVE

127

## REAGENTS

(a) *Benedict's soln* (qualitative).—Dissolve 17.3 g of Na citrate and 10 g of anhydrous  $\text{Na}_2\text{CO}_3$  in ca 80 ml of hot  $\text{H}_2\text{O}$ ; dissolve 1.73 g of crystal  $\text{CuSO}_4$  in 10 ml of  $\text{H}_2\text{O}$ . Filter the alkaline citrate soln, add the  $\text{CuSO}_4$  soln slowly, with constant stirring, and dilute with  $\text{H}_2\text{O}$  to 100 ml.

(b) *Sodium hydroxide soln*.—Approximately 2 N. 10 g of NaOH in 100 ml of  $\text{H}_2\text{O}$ .

128

## PREPARATION OF SAMPLE

(a) *Cream cheese or creamed cottage cheese*.—Transfer 100 g of cheese to 500 ml casserole, warm on steam bath, and macerate with petroleum benzin. Decant benzin and repeat treatment until benzin is colorless. Warm the defatted cheese on steam bath to remove residual petroleum benzin, add 100 ml of hot  $\text{H}_2\text{O}$ , and bring to boil, stirring continuously. Remove casserole from flame, add 4 or 5 drops of ammonia, mix thoroly, and transfer to 250 ml centrifuge bottle or nursing bottle. Neutralize with acetic acid, using litmus paper as indicator, and add 4 or 5 drops in excess. Shake thoroly and centrifuge 10–20 min. Separate liquid either by decantation or by use of a pipet-guard<sup>36</sup> and proceed as directed under 129.

(b) *Cream cheese that does not separate distinctly into layers upon centrifuging or that separates into a top layer of curd and fat, a middle aqueous layer, and a bottom layer of curd*.—Separate the aqueous gum soln by filtering under vacuum thru a coarse filter, using 11 cm Büchner funnel. Proceed as directed under 129.

(c) *Cottage cheese (uncreamed)*.—Omitting the treatment with petroleum benzin, proceed as directed under (a), beginning, "add 100 ml of hot  $\text{H}_2\text{O}$ ."

129

## SEPARATION OF GUM

Evaporate to ca 40 ml the separated gum soln obtained by centrifuging or filtering (disregard precipitate formed during concentration), add 10 ml of trichloroacetic acid soln (50 g/100 ml), and hold at 70° until the precipitate coagulates, avoiding prolonged heating, which will hydrolyze the gum. Cool, centrifuge, and filter. (Pinch of filter-cel may be added to aid clarification.)

Add 4 volumes of alcohol to filtrate and allow mixture to stand 2–3 hours, if necessary to coagulate separated gum.

130

## DETECTION OF GUM

Centrifuge mixture, decant supernatant liquid, and wash with 70% alcohol. Centrifuge, and again decant or filter. Dissolve residue in minimum quantity of hot  $\text{H}_2\text{O}$ , and reprecipitate with 4 volumes of alcohol plus 2 or 3 drops of acetic acid. (This reprecipitation removes traces of sugars occluded by the first precipitation.)

Allow precipitate to coagulate, centrifuge, and decant. Dissolve residue in minimum quantity of  $\text{H}_2\text{O}$ , transfer to 50 ml beaker, and evaporate to 5 ml. Add 2 ml of HCl. Boil 30–60 seconds. Transfer 1 ml of the hydrolyzed sugar soln to test tube, neutralize with the NaOH soln, using litmus paper as an indicator, remove the litmus paper, add 5 ml of the Benedict soln, and boil vigorously 1–2 min. Allow to cool spontaneously. A voluminous precipitate appearing on cooling, which may be green, yellow, or red, caused by reducing sugars formed by hydrolysis of the gums, indicates presence of gums.

Add an equal volume of HCl to remaining hydrolyzed gum soln, boil 30–60 seconds and add 2 or 3 crystals of phloroglucinol. A deep amber or cherry red color at this point confirms the presence of gums.

## GELATIN IN COTTAGE CHEESE

131

*Qualitative Test—Official*

Mix thoroly 5 g of sample with 10 ml of  $H_2O$  at 50–60° and 5 ml of  $Hg(NO_3)_2$  soln. Shake, allow to stand 5 min., and filter thru medium fast retentive paper. To filtrate add 5 ml more of the  $Hg(NO_3)_2$  soln and test as directed in 29, using filtrate so obtained. See also note under 29.

## ICE CREAM (PLAIN)

132

## PREPARATION OF SAMPLE—OFFICIAL

Allow sample to soften at room temp. Because melted butter fat tends to separate out and rise to surface, it is not advisable to soften the ice cream by heating on water bath or over flame. Mix thoroly by stirring with spoon or egg beater or by pouring back and forth between beakers.

133

## NITROGEN—OFFICIAL

Proceed as directed in II, 23, using 4–5 g sample. Percentage of  $N \times 6.38 =$  percentage "protein."

## FAT

134

*Roesse-Gottlieb Method—Official*

Weigh 4 g of thoroly mixed sample into small dry beaker, add 3 ml of  $H_2O$ , mix thoroly, and transfer to Röhrig tube or similar apparatus, washing out remaining portion with aid of an additional 3 ml of  $H_2O$ . Add 2 ml of  $NH_4OH$ , mix thoroly and heat in water bath at 60°. Proceed as directed under 20, beginning "Add 10 ml of alcohol and mix well."

135

## COLORING MATTERS—TENTATIVE

Curdle 150–200 g of the melted sample by adding an equal volume of  $H_2O$  and 10–20 ml of acetic acid. Heat mixture to 70–80°, stirring meanwhile, and allow to cool. Continue as directed under 31 and 105 and under XXI, particularly 3 and 21 for detection of oil-soluble coal tar dyes and annatto.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 15, 643 (1932); 19, 93 (1936).
- <sup>2</sup> Ibid., 10, 261 (1927); 13, 42 (1930).
- <sup>3</sup> J. pharm. chim., 9, 108 (1884).
- <sup>4</sup> Browne, Handbook of Sugar Analysis, 1912, p. 252.
- <sup>5</sup> Z. Nahr. Genussm., 9, 531 (1905).
- <sup>6</sup> J. Assoc. Official Agr. Chem., 8, 4 (1924); 270, 471 (1925).
- <sup>7</sup> J. Am. Chem. Soc., 26, 1195 (1904).
- <sup>8</sup> Z. öfentl. chem., 9, 173 (1903).
- <sup>9</sup> Chem. Ztg., 32, 617 (1908).
- <sup>10</sup> U. S. Dept. Agr. Bur. Chem., Bull. 132, p. 124 (1910).
- <sup>11</sup> J. Ind. Eng. Chem., 13, 198 (1921); J. Assoc. Official Agr. Chem., 5, 172, 470, 484 (1922); 6, 424, 429 (1923).
- <sup>12</sup> J. Am. Chem. Soc., 22, 207 (1900); J. Assoc. Official Agr. Chem., 19, 92 (1936).
- <sup>13</sup> J. Assoc. Official Agr. Chem., 13, 237 (1930); 19, 93 (1936); Am. J. Public Health, 4, 50 (1914).
- <sup>14</sup> J. Assoc. Official Agr. Chem., 21, 82 (1938); J. Dairy Research, 6, 191 (1935); 25th Ann. Rpt. Intern. Assoc. Milk Sanitarians, 1936, 15–39.
- <sup>15</sup> J. Assoc. Official Agr. Chem., 19, 242 (1936); 20, 78, 213 (1937); 21, 90 (1938); 22, 81, 468 (1939).
- <sup>16</sup> Ibid., 10, 34 (1927).
- <sup>17</sup> J. pharm. chim., 15, 211 (1902); Z. Nahr. Genussm., 5, 726 (1902).

- <sup>18</sup> J. Assoc. Official Agr. Chem., 18, 77 (1935).
- <sup>19</sup> Ibid., 11, 43 (1928).
- <sup>20</sup> Ibid., 6, 435 (1923).
- <sup>21</sup> Ibid., 15, 75 (1932); 19, 93 (1936).
- <sup>22</sup> Ibid., 12, 238 (1929).
- <sup>23</sup> Ibid., 18, 78 (1935).
- <sup>24</sup> Ibid., 21, 84 (1938).
- <sup>25</sup> U. S. Dept. Agr. Farmers Bull. 131, p. 6; Bur. Chem. Circ. 19, rev., p. 1 (1905).
- <sup>26</sup> J. Assoc. Official Agr. Chem., 20, 93 (1937); 22, 76, 495 (1939).
- <sup>27</sup> Ibid., 5, 498 (1922); 18, 57 (1935).
- <sup>28</sup> Ibid., 18, 401 (1935); 20, 70 (1937); 21, 82 (1938).
- <sup>29</sup> Ibid., 21, 82 (1938).
- <sup>30</sup> Ibid., 11, 40 (1928).
- <sup>31</sup> Ibid., 17, 66 (1934).
- <sup>32</sup> Ibid., 10, 264 (1927); 11, 41 (1928).
- <sup>33</sup> Ibid., 15, 75 (1932); 17, 66 (1934).
- <sup>34</sup> Ibid., 13, 243 (1930).
- <sup>35</sup> Ibid., 18, 79 (1935); 20, 527 (1937).
- <sup>36</sup> Ibid., 20, 529 (1937).

## XXIII. EGGS AND EGG PRODUCTS

1

### COLLECTION AND PREPARATION OF SAMPLE<sup>1</sup>—TENTATIVE

No simple rules can be made for the collection of a sample representative of the average of any particular lot of egg material as conditions may differ widely. Experienced judgment must be used in each instance. If large lots are under examination, it is best to draw a number of samples for separate analyses rather than to attempt to get one composite representative sample.

(a) *Liquid eggs*.—Secure representative container or containers. Mix contents of a container thoroly and draw ca 300 g. A long-handled dipper or ladle serves well. Keep sample in hermetically sealed jar in cool place. Report odor and appearance.

(b) *Frozen eggs*.—Secure representative container or containers. Examine contents as to odor and appearance. The condition of contents can be determined best by boring a hole to center of a container with auger and noting odor as the auger is withdrawn. If impossible to secure individual containers, samples may consist of the composite of the borings from the contents of each container. Take borings midway between center and circumference of the top of the can from at least 3 widely separated parts and extend them as near to bottom of can as possible. Collect ca 300 g of the sample. Keep sample in hermetically sealed jar in cool place and in frozen state if possible. Before analyzing warm the sample in bath held below 50° and mix well.

(c) *Dried eggs*.—Secure representative container or containers. For small packages, take entire parcel or parcels for the sample. For boxes and barrels, remove top layer to depth of ca 6" with flour scoop or other convenient instrument. Draw small quantities of sample totaling 300–500 g from accessible parts of container and place in hermetically sealed jar. Report odor and appearance. Prepare sample for analysis by mixing 3 times thru a domestic flour sifter to assure complete breaking up of lumps. Keep in hermetically sealed jar in cool place.

(d) *Flaked and drum-dried eggs*.—Collect sample as directed for powdered dried eggs. Report odor and appearance. Prepare albumin samples for analysis by grinding in mill to pass entirely thru 60-mesh sieve, and whole egg and yolk samples to pass entirely thru 20-mesh sieve or as fine as is practicable. Keep in hermetically sealed jar in cool place.

### TOTAL SOLIDS

#### *Vacuum Method<sup>2</sup>—Official*

2

### APPARATUS

*Vacuum oven*.—Connected with a pump to maintain partial vacuum in oven with pressure equivalent to 25 mm or less of Hg and provided with thermometer passing into oven with bulb near samples. Connect an H<sub>2</sub>SO<sub>4</sub> gas-drying bottle to oven for admitting dry air to release vacuum.

3

### DETERMINATION

(a) *Liquid eggs*.—Weigh accurately by difference by means of weighing buret ca 5g of the sample 1(a) or (b) in a covered dish that previously has been dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Remove cover and drive off most of H<sub>2</sub>O by heating on steam bath. Replace cover loosely and complete drying in vacuum oven as directed under (b).

(b) *Dried eggs*.—Weigh ca 2 g of the sample, 1(c), in covered dish that previously

has been dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat at 98–100° to constant weight (ca 5 hours) in vacuum oven. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover of dish, transfer to desiccator containing fresh efficient desiccant, and weigh soon after room temp. is attained. Report weight of egg residue as percentage total solids.

#### ORGANIC AND AMMONIACAL NITROGEN<sup>3</sup>—OFFICIAL

4

##### PREPARATION OF SAMPLE

(a) *Liquid eggs*.—Weigh 2–3 g of well-mixed sample, 1(a) or (b), by difference into 500 ml Kjeldahl flask.

(b) *Dried eggs*.—Transfer ca 1 g of sample, 1(c), accurately weighed, to 500 ml Kjeldahl flask.

5

##### DETERMINATION

Determine N as directed under II, 21, 22, or 23. (Complete digestion is accomplished most rapidly by Kjeldahl-Gunning-Arnold method, 23.) Distil the  $\text{NH}_3$  into 30–50 ml of 0.1 *N* standard acid.

#### WATER-SOLUBLE NITROGEN AND CRUDE ALBUMIN NITROGEN<sup>4</sup>—OFFICIAL FOR LIQUID EGGS, TENTATIVE FOR DRIED EGGS

6

##### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately, by difference, into 250 ml volumetric flask containing 150 ml of  $\text{H}_2\text{O}$ , ca 10 g of well-mixed sample, 1(a) or (b), and mix gently. Add 5 ml of 0.01 *N* acetic acid for each gram of egg substance, fill to mark with  $\text{H}_2\text{O}$ , shake gently, and filter thru 18½ cm folded filter, covering filter with watch-glass during filtration. If filtrate is cloudy, allow filtration to continue until drops of filtrate become clear, change receiving container, return cloudy filtrate to filter and proceed as directed under 7.

(b) *Dried eggs*.—From the sample, 1(c), transfer 1 g of whites, 3 g of whole eggs or 5 g of yolks into an 8 ounce nursing bottle, add 50 ml of petroleum benzin, mix gently, centrifuge, and decant solvent. Repeat treatment with petroleum benzin. Place bottle on its side, rolling it occasionally until residue is dry, and break up the dry residue with glass rod having a flattened end. Add 100 ml of  $\text{H}_2\text{O}$  (slowly at first with gentle mixing until sample disintegrates), then add 5 ml of 0.01 *N* acetic acid for each gram of egg substance and sufficient  $\text{H}_2\text{O}$  to make a total of exactly 200 ml of  $\text{H}_2\text{O}$  and acid. Mix gently and allow to stand 2 hours, continuing mixing at intervals. Centrifuge, filter, and proceed as directed under 7.

7

##### DETERMINATION

(a) *Water-soluble nitrogen*.—Transfer 50 ml of the clear prepared filtrate into 500 ml Kjeldahl flask, and determine N as directed under II, 23, using  $\text{HgO}$ . Calculate the N and report as percentage of water-soluble N.

(b) *Crude albumin nitrogen*.—Transfer 100 ml of the clear prepared filtrate to 200 ml volumetric flask, add 15 ml of NaCl soln (28 g NaCl diluted to 300 ml), fill nearly to mark with alcohol, and mix. Cool to room temp., fill to mark with alcohol, shake, and allow to stand overnight. Filter, transfer 100 ml of filtrate to 500 ml Kjeldahl flask, and determine N as directed under II, 23, using  $\text{HgO}$ . Calculate percentage of N, subtract it from percentage of water-soluble N, and report difference as percentage of crude albumin N.



FAT BY ACID HYDROLYSIS<sup>1</sup>—TENTATIVE

8

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—From the well-mixed sample, 1(a) or (b), weigh accurately by difference into fat extraction tube (Mojonnier tube is convenient) ca 2 g of yolks, or 3 g of whole eggs, or 5 g of whites. Add slowly with vigorous shaking 10 ml of HCl, set tube in water bath heated to 70°, bring to boiling, and continue heating at boiling for 30 min., shaking tube with care at 5 min. intervals. Remove tube from water bath, add H<sub>2</sub>O nearly to fill lower bulb of tube, and cool to room temp.

(b) *Dried eggs*.—Transfer 1 g of the well-mixed sample to fat extraction tube; add slowly, washing down any egg particles adhering to sides of tube, 10 ml of HCl (4+1); and proceed as directed in (a).

9

## DETERMINATION

To the extraction tube containing the treated sample, 8, add 25 ml of ethyl ether, and mix. Add 25 ml of redistilled petroleum benzin (b.p. below 60°), mix, and allow to stand until solvent layer is clear. Proceed as directed in XX, 11, beginning "Decant as much as possible," but omitting filtration.

LIPOIDS AND LIPOID PHOSPHORIC ACID (P<sub>2</sub>O<sub>5</sub>)<sup>2</sup>—TENTATIVE

10

## REAGENTS

(a) *Mixed solvent*.—Equal volumes of CHCl<sub>3</sub> and absolute alcohol.

(b) *Alcoholic sodium hydroxide*.—Prepare a saturated soln free from carbonates by dissolving 100 g of NaOH in 100 ml of H<sub>2</sub>O. Allow mixture to stand until clear, or filter thru a hard filter paper that has been soaked in alcohol (5 ml of the NaOH soln contains ca 4 g of NaOH). Dissolve 50 ml of this soln in 900 ml of alcohol and dilute with alcohol to 1 liter.

11

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately by difference ca 4 g of the well-mixed sample, (a) or (b), into 100 ml flask, add very slowly (dropwise) from a pipet, 25 ml of the mixed solvent, shaking constantly until proteins become coagulated and then thoroly broken up. Add 60–65 ml more of solvent and allow to stand 1 hour, shaking at 5 min. intervals. Fill to mark with solvent, shake, and allow mixture to stand until clear.

(b) *Dried eggs*.—Transfer 2 g of the well-mixed sample, 1(c), to 100 ml volumetric flask, add 85–90 ml of the mixed solvent, and allow to stand 1 hour, mixing at 5 min. intervals. Proceed as directed in (a).

12

## DETERMINATION

(a) *Lipoids*.—Transfer 50 ml aliquot to 150 ml beaker and evaporate extract to dryness on steam bath. (An electric fan or a gentle blast of dry air may be used to hasten evaporation.) Place beaker in oven at 100° for 5–10 min. to remove any remaining moisture. Dissolve dry extract in 5–10 ml of CHCl<sub>3</sub>, and filter soln into weighed 100 ml Pyrex beaker thru pledget of cotton packed into stem of funnel, transferring all soluble extract from bottom and sides of beaker by means of CHCl<sub>3</sub>. Finally wash funnel and stem tip (filtrate should be clear). Evaporate the CHCl<sub>3</sub> on steam bath, and dry beaker and contents in oven at 100° to constant weight (ca 90 min.). Allow beaker to stand in air to constant weight (ca 30 min.), weigh, and report percentage of lipoids.

(b) *Lipoid phosphoric acid* (P<sub>2</sub>O<sub>5</sub>).—Dissolve dried lipoids in 2–3 ml of CHCl<sub>3</sub>,

add 10–20 ml of the alcoholic NaOH soln, evaporate to dryness on steam bath, using care to avoid spattering, and place beaker in oven at 100° for 30 min. to remove any remaining moisture. Transfer beaker while hot to electric muffle heated to 500° (faint redness), and allow it to remain at this temp. 1 hour. Cool, add a few drops of H<sub>2</sub>O, break up charge with glass rod having a flattened end, cover beaker with watch-glass, add slowly 5 ml of HNO<sub>3</sub> (1+3), mix, wash watch-glass and filter, collecting filtrate in 300 or 500 ml Erlenmeyer flask. Thoroughly wash charred material and filter paper with H<sub>2</sub>O.

In filtrate determine phosphoric acid (P<sub>2</sub>O<sub>5</sub>) as directed under II, 12, using 20–50 ml of the molybdate soln. Report percentage of lipid P<sub>2</sub>O<sub>5</sub> in the eggs.

#### TOTAL PHOSPHORIC ACID (P<sub>2</sub>O<sub>5</sub>)<sup>7</sup>—OFFICIAL

13

##### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—From the well-mixed sample, 1(a) or (b), weigh accurately, by difference, into 250 ml low-form Pyrex beaker, ca 2 g of yolk, 4 g of whole eggs, or 10 g of whites. Add 20 ml of 10% Na<sub>2</sub>CO<sub>3</sub> soln and evaporate to dryness on electric hot plate or overnight at 100–105°. Transfer beaker while hot to electric muffle heated to 500° (faint redness), and allow it to remain at this temp. 1 hour. Cool, add a few drops of H<sub>2</sub>O, break up charge with glass rod having a flattened end, cover beaker with watch-glass, add slowly while stirring 10 ml of HNO<sub>3</sub> (1+3), mix, wash watch-glass, and filter, collecting filtrate in 300 or 500 ml Erlenmeyer flask. Thoroughly wash charred material and filter with H<sub>2</sub>O.

(b) *Dried eggs*.—Transfer 1 g of the well-mixed sample, 1(c), to 150 ml low-form Pyrex beaker, add 20 ml of 10% Na<sub>2</sub>CO<sub>3</sub> soln, and proceed as directed under (a).

14

##### DETERMINATION

In the prepared filtrate determine P<sub>2</sub>O<sub>5</sub> as directed under II, 12, using 40–50 ml of the molybdate soln. Report as total P<sub>2</sub>O<sub>5</sub>.

15

#### CHLORINE<sup>8</sup>—OFFICIAL

(a) *Liquid eggs* (in absence of added salt).—From the well-mixed sample, 1(a) or (b), weigh accurately, by difference, into 150 ml low-form Pyrex beaker ca 4 g of yolk, 7 g of whole eggs, or 10 g of whites; add 20 ml of 10% Na<sub>2</sub>CO<sub>3</sub> soln, mix, and evaporate to dryness on electric hot plate or overnight at 100°. Transfer beaker while hot to electric muffle heated to 500° (faint redness), and allow to remain at that temp. 1 hour. Cool, add a few drops of H<sub>2</sub>O, and break up charge with glass rod. Add 50 ml of H<sub>2</sub>O, cover beaker with watch-glass, add slowly 20 ml of HNO<sub>3</sub> (1+3), wash watch-glass, mix, filter, and wash charred material and filter thoroughly with H<sub>2</sub>O. Proceed as directed in one of the following alternatives:

(1) To combined filtrate and washings add known volume of 0.1 N AgNO<sub>3</sub> in slight excess and proceed as directed in XII, 37.

(2) Collect the filtrate and washings in 250 ml flask, keeping total volume of filtrate to 180 ml or less. Add known volume of 0.1 N AgNO<sub>3</sub> in slight excess and make to volume. Filter, and using an aliquot of the filtrate, proceed as directed in XII, 37, beginning “add 5 ml of the ferric indicator.”

(b) *Liquid eggs* (in presence of added salt).—From the well-mixed sample, 1(a) or (b), weigh 1–2 g accurately, by difference, into 150 ml low-form Pyrex beaker, and proceed as directed under (a).

(c) *Dried eggs*.—From the well-mixed sample, 1(c), transfer to 150 ml low-form Pyrex beaker, 2 g of whole eggs or yolks, or 1 g of whites, and proceed as directed under (a).

DEXTROSE AND SUCROSE<sup>2</sup>—OFFICIAL

16

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately, by difference, ca 25 g of the well-mixed sample, 1(a) or (b), into 250 ml volumetric flask containing 1 g of  $\text{CaCO}_3$  and 50 ml of 5%  $\text{NaCl}$ . Add with continuous mixing 130 ml of alcohol. Allow to stand a few minutes for gas bubbles to rise to surface, cool to room temp., fill to mark with  $\text{H}_2\text{O}$ , shake, and filter (18.5 cm folded filter). Transfer 150 ml of filtrate to 250 ml beaker, evaporate to 20–30 ml to remove alcohol, cool, and wash with  $\text{H}_2\text{O}$  into 100 ml volumetric flask, holding the volume to 80–90 ml; add dry powdered phosphotungstic acid in small quantities in slight excess to precipitate any protein, mix, let stand a few minutes for gas bubbles to rise to surface, fill to mark with  $\text{H}_2\text{O}$ , shake, and filter. To filtrate, add in very small portions sufficient dry powdered  $\text{KCl}$  to precipitate any excess phosphotungstic acid, filter if necessary, and test filtrate for complete precipitation.

To correct for error due to volume occupied by precipitate in samples containing added sucrose, repeat determination weighing same quantity of sample into 500 ml volumetric flask containing 1 g of  $\text{CaCO}_3$  and 100 ml of 5%  $\text{NaCl}$  soln. Add, with continuous mixing, 260 ml of alcohol. Allow mixture to stand a few minutes for gas bubbles to rise to surface, cool to room temp., fill to mark with  $\text{H}_2\text{O}$ , shake, and filter thru 18.5 cm folded filter. Transfer 300 ml of filtrate to 400 ml beaker, evaporate to 20–30 ml, and proceed as directed above. To obtain amount of sucrose subtract percentage of sucrose obtained in 250 ml dilution determination from twice percentage obtained in 500 ml dilution determination.

(b) *Dried eggs*.—From the well-mixed sample, 1(c), transfer to 250 ml volumetric flask containing 1 g of  $\text{CaCO}_3$  and 50 ml of 5%  $\text{NaCl}$  soln 2.5 g of whites, or 10 g of yolks or whole eggs, and allow to stand 1 hour, mixing at 5 min. intervals. Add with continuous mixing 130 ml of alcohol, and proceed as directed under (a), beginning with the 3rd sentence.

17

## DETERMINATION

*Reducing sugars direct*.—Transfer 25 ml of the prepared filtrate to 400 ml beaker, and proceed as directed under XXXIV, 38. Report as percentage of dextrose.

*Reducing sugars invert*.—Transfer 50 ml of the prepared filtrate to 100 ml volumetric flask, and invert the sucrose as directed in XXXIV, 24(b) or (c). Neutralize with  $\text{NaOH}$  soln, cool to room temp., and fill to mark with  $\text{H}_2\text{O}$ . Transfer 50 ml (or less) to 400 ml beaker, and proceed as directed under XXXIV, 38. Deduct percentage of invert sugar obtained before inversion from that obtained after inversion, multiply difference by 0.95, and report as percentage of sucrose.

GLYCEROL<sup>10</sup>*Qualitative Test—Tentative*

18

## REAGENT

*Fuchsin-sulfite soln*.—Dissolve 0.2 g of fuchsin in 120 ml of hot  $\text{H}_2\text{O}$ , cool, and add soln of 2 g of anhydrous  $\text{Na}_2\text{SO}_3$  in 20 ml of  $\text{H}_2\text{O}$ , then 2 ml of  $\text{HCl}$ . Dilute soln with  $\text{H}_2\text{O}$  to 200 ml and allow to stand 1 hour.

19

## DETECTION

Add 20 ml of alcohol to ca 5 g of sample in an Erlenmeyer or beaker flask, shake vigorously, and filter thru 12.5 cm fluted filter paper. Evaporate filtrate rapidly

until no odor of alcohol is perceptible, cool, and add 3–4 drops of  $\text{H}_2\text{O}$  and then 10–15 ml of anhydrous ether. Mix solns carefully, allow to separate, and pour off as much as possible of the ether layer, disregarding cloudiness in this layer. Shake well with two 10 ml portions of anhydrous ether, pouring off ether carefully in each case. (Volume of aqueous soln should not be less than 0.4–0.5 ml.) Evaporate remaining liquid on steam bath to 0.1–0.2 ml. Cool, and add 15 ml of mixture of equal volumes of absolute alcohol and  $\text{CHCl}_3$ . Cool, shake, and allow mixture to stand 5 min. to permit crystallization of sugar. Shake, and filter thru fluted filter paper into 6×1" test tube (hard glass). Evaporate filtrate rapidly (small flame in front of fan is convenient) until no odor of  $\text{CHCl}_3$  or alcohol is perceptible. Add several grams of powdered  $\text{K}_2\text{SO}_4$  and insert a stopper with a glass tube leading into 2 ml of  $\text{H}_2\text{O}$  in a test tube immersed in ice  $\text{H}_2\text{O}$ . Heat with small flame until frothing ceases and contents of tube are liquid. Remove receiver, add immediately 4–5 drops of the fuchsin-sulfite reagent, and warm to room temp. In presence of glycerol a strong pink color (due to acrolein) develops within 1 min. and becomes a deep violet within 5 min.

*Quantitative Method—Tentative*

(Not applicable in presence of sugars.)

## 20

## REAGENTS

- (a) *Mercuric nitrate soln.*—See XXII, 83
- (b) *Diphenylamine indicator soln.*—See XXXIII, 73(d).
- (c) *Phosphoric acid-sulfuric acid soln.*—Add 150 ml of  $\text{H}_2\text{SO}_4$  and 150 ml of sirupy phosphoric acid to 500 ml of  $\text{H}_2\text{O}$  and dilute with  $\text{H}_2\text{O}$  to 1 liter.
- (d) *Potassium dichromate soln.*—See XXXIII, 73(a).
- (e) *Ferrous ammonium sulfate soln.*—See XXXIII, 73(c).
- (f) *Basic lead acetate soln.*—See XXXIV, 19(a).
- (g) *Thymol blue indicator soln.*—Dissolve 0.1 g of thymol blue in 21.55 ml of 0.01 N NaOH soln and dilute to 250 ml with  $\text{H}_2\text{O}$ .

## 21

## DETERMINATION

Weigh by difference ca 5 g of sample into 100 ml volumetric flask containing 50–75 ml of  $\text{H}_2\text{O}$ , mix well, add 2 ml of the  $\text{Hg}(\text{NO}_3)_2$  soln, again mix well, and make up to mark with  $\text{H}_2\text{O}$ . Mix, and transfer contents of flask to 8 oz. centrifuge bottle. Add 5 g of light magnesium carbonate, stopper, and shake vigorously for several minutes. Centrifuge 1–2 min., pour off supernatant liquid thru fluted filter, and transfer 75 ml to centrifuge bottle. Add 50 ml of the basic lead acetate soln and then 50 ml of 2 N KOH, mix, and add a drop of the thymol blue indicator soln. If surface of liquid does not turn deep blue, add more alkali. Let stand 5–10 min., centrifuge until clear, and pour off liquid into 250 ml volumetric flask. Shake residue with 20 ml of  $\text{H}_2\text{O}$ , centrifuge, and add washings to flask. Add 2 drops of thymol blue to soln and add  $\text{H}_2\text{SO}_4$  (1+1) until distinct pink color is obtained. Make up to mark with  $\text{H}_2\text{O}$ , mix, and filter thru dry filter paper. Transfer 200 ml of filtrate to 400 ml beaker, evaporate rapidly to 75 ml, and finish evaporation on water bath or slow hot plate to 35–40 ml. Transfer liquid to 50 ml volumetric flask and make up to volume with  $\text{H}_2\text{O}$ . Transfer 25 ml to 250 ml volumetric flask, and add 20 ml of the  $\text{K}_2\text{Cr}_2\text{O}_7$  soln and 25 ml of  $\text{H}_2\text{SO}_4$ . Run a blank, using 20 ml of the  $\text{K}_2\text{Cr}_2\text{O}_7$  soln and 25 ml of  $\text{H}_2\text{O}$ . Heat in *boiling* water bath exactly 20 min. Cool, dilute to volume, mix, and transfer some of soln to buret. Pipet 20 ml of the  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$  soln into beaker, add 100 ml of  $\text{H}_2\text{O}$ , 15 ml of the phosphoric-sulfuric acid soln and just 3 drops of the diphenylamine soln, and titrate with the  $\text{K}_2\text{Cr}_2\text{O}_7$  soln. When the green

color has changed to a blue-gray, add the  $K_2Cr_2O_7$  soln slowly, swirling after each drop. The end point is reached when addition of 1 drop of the  $K_2Cr_2O_7$  soln changes the color to deep violet. Subtract 0.05 ml from reading to correct for oxidation of indicator.

$$\text{Percentage glycerol} = \frac{100(a-b)}{Wa}, \text{ in which}$$

$a$  = ml of  $K_2Cr_2O_7$  titrated in unknown soln,  
 $b$  = ml of  $K_2Cr_2O_7$  titrated in blank soln, and  
 $W$  = weight of sample in grams.

#### ACIDITY OF ETHER EXTRACT

(Not applicable to egg white.)

#### Method I<sup>11</sup>—Official

### 22

#### REAGENTS

(a) *Benzene*.—Use best available quality of benzene. If it is not neutral, titrate 50 ml with the 0.05 *N* Na ethylate, reagent (b), and correct subsequent results accordingly.

(b) *Sodium ethylate*.—0.05 *N*. Dissolve a piece of metallic Na, ca 1 ml in volume, in 800 ml of absolute alcohol. Titrate 10 ml of 0.1 *N* HCl with this soln and add calculated volume of absolute alcohol to make soln 0.05 *N*. Ascertain normality factor by titration against 0.1 *N* HCl on day soln is used.

### 23

#### DETERMINATION

(a) *Dried eggs*.—Weigh to nearest milligram ca 2 g of powdered sample, 1(c), in tared Al dish ca 63 mm in diameter and dry at 55° under pressure not exceeding 125 mm of Hg. Weigh at end of 2 hours and continue drying for half-hour periods to constant weight. Carefully transfer dried residue to a 12.5 cm hardened filter paper, fold paper once, place it on a 15 cm qualitative filter paper, and roll papers and contents into cylinder that will fit snugly into extraction tube, folding in one end of cylinder to prevent loss of material. Extract with anhydrous ether, preferably in Knorr apparatus. (An asbestos plug is not needed in extraction tube, and if extractor is working rapidly, 3 hours is sufficient for proper extraction.) Evaporate ether from extraction flask, dry extract 1 hour at 55° under pressure not exceeding 125 mm, and weigh to nearest milligram. Dissolve extract in 50 ml of benzene, add 3 to 4 drops of phenolphthalein indicator, and titrate with the Na ethylate soln. The end point is reached when yellow color changes to orange. Report as ml of 0.05 *N* Na ethylate required per g of ether extract.

(b) *Liquid eggs*.—Weigh to nearest milligram in tared Pb dish ca 5 g of sample, 1(a) or (b), and dry as directed under (a). Weigh after ca 5 hours and continue drying for 1 hour periods to constant weight. To prepare dried residue for extraction with anhydrous ether, place dish upon 12.5 cm hardened filter paper, cut sides of dish thru at 4 equidistant points, and flatten down. Place another similar filter paper on top of dish and its contents and roll papers and dish into cylinder as directed under (a). Proceed as directed under (a).

#### Method II<sup>2</sup>—Rapid Method—Tentative

(For liquid eggs.)

### 24

#### REAGENTS

(a) *Salt soln*.—Dissolve 10 g of NaCl in ca 50 ml of  $H_2O$ , add 30 ml of alcohol, and dilute to 100 ml with  $H_2O$ .

(b) *Benzene*.—See 22(a).

(c) *Sodium ethylate*.—See 22(b).

25

## DETERMINATION

Weigh 10 g of mixed whole liquid egg or 5 g of liquid yolks and transfer to suitable centrifuge bottle with 40 ml of the NaCl soln. Shake gently until the egg is thoroly mixed with the NaCl soln. Add 50 ml of ethyl ether followed by 50 ml of petroleum benzin, stopper bottle, and shake gently but thoroly until lipoids are extracted. (Solvent layer is yellow when lipoids are extracted. If the solvent layer is not yellow, shake more vigorously.) Centrifuge to separate liquids. If bad emulsion has formed, add 10 ml of ethyl alcohol, shake gently, and centrifuge again. Remove ether layers by carefully pouring off or blowing off with wash bottle arrangement, or by any other method that will separate the solvent layer. Repeat extraction, using 30 ml each of ether and petroleum benzin, and add to first solvent shakeout. If only acidity of the ether extract is to be determined, enough lipoids will be obtained by two extractions for titration purposes. If complete extraction is desired, repeat shaking with ca 50 ml of mixed ethers, centrifuging, etc., until ether layer is colorless after separation.

Evaporate mixture of solvents from first two extracts in suitable dish on steam bath. When solvent is removed, add 5 ml of absolute alcohol and evaporate again on steam bath to aid in removal of moisture. Dissolve residual extract in small quantity of  $\text{CHCl}_3$ , filter into tared beaker, and wash dish and filter with  $\text{CHCl}_3$ . Evaporate off the  $\text{CHCl}_3$  on steam bath and continue heating a few minutes after the  $\text{CHCl}_3$  is removed. Dry beaker with a towel. Cool, and weigh.

Proceed as directed under 23(a), beginning "Dissolve extract in 50 ml of benzene."

AMMONIA NITROGEN<sup>13</sup>—TENTATIVE

(For liquid eggs.)

26

## APPARATUS

Apparatus consists of a train, items (a), (b), (c), and (d), each provided with a two-holed rubber stopper so connected as to permit proper passage of air, which is supplied by a pump with pressure of 10 lbs. per sq. in. Compensate for pulsations of pump to assure delivery of steady pressure by placing tank of sufficient size between pump and bottle (a). Suction may be used to draw air thru train but pressure is preferable.

(a) *Wash bottle*.—Contains  $\text{H}_2\text{SO}_4$  (ca 35%) for removal of ammonia from the air supply. The inlet tube is provided with stopcock to regulate the air supply.

(b) *Trap*.—To prevent mechanical transfer of  $\text{H}_2\text{SO}_4$  into (c).

(c) *Aerating cylinder*.—About 50 mm in diameter and 350 mm high. The inlet tube extends to within  $\frac{1}{2}$ " of bottom. The outlet tube is provided with trap containing cotton or glass wool to prevent liquid from being carried over.

(d) *Bottle*.—Wide-mouthed, 8 oz. Inlet tube terminates in small bulb punctured with a few small holes to expedite ammonia absorption.

27

## DETERMINATION

Weigh ca 25 g of sample, 1(a) or (b), in convenient container. Pour as much as possible of this material into aeration cylinder (c) and transfer remainder by means of four 25 ml portions of ammonia-free  $\text{H}_2\text{O}$ , stirring each time with policeman to remove any egg adhering to sides of weighing vessel. Add 75 ml of alcohol, mix well.

and let stand for 15 min. Add ca 1 g of NaF, 5 ml of 20% soln of  $\text{Na}_2\text{CO}_3$ , and 1 ml of kerosene, or if necessary let stand overnight before adding the  $\text{Na}_2\text{CO}_3$ .

Connect train and aerate thru 10 ml of 0.02 *N*  $\text{H}_2\text{SO}_4$  (if sample has bad odor, more acid may be required), 2 drops of methyl red indicator (saturated soln in alcohol), and ca 75 ml of ammonia-free  $\text{H}_2\text{O}$  in the receiving bottle (d).

Use as rapid a current of air as possible without splashing the egg soln into the trap following cylinder (c). Determine time to aerate as follows: In a duplicate train measure 20 ml of  $\text{NH}_4\text{Cl}$  or  $(\text{NH}_4)_2\text{SO}_4$  soln (ca 5% stronger than 0.02 *N*) into aeration cylinder (c) and 20 ml of 0.02 *N*  $\text{H}_2\text{SO}_4$ , 75 ml of  $\text{H}_2\text{O}$ , and several drops of methyl red indicator into receiving bottle (d). Aerate until soln in receiving bottle changes color, noting time required. The sample should be aerated 30 min. longer than this time.

Titrate excess of acid in cylinder (d) with 0.02 *N* NaOH (free from  $\text{CO}_2$ ). Express results as mg of ammonia nitrogen per 100 g of sample. Correct results for a blank determination on apparatus and reagents.

## 28

## EXTRACTION AND IDENTIFICATION OF ADDED COLOR

Proceed as directed under **XX, 99**.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., **8**, 599 (1925); **9**, 56 (1926).
  - <sup>2</sup> Ibid., **8**, 600 (1925); **9**, 56, 354 (1926); **14**, 85, 395 (1931).
  - <sup>3</sup> Ibid., **8**, 601 (1925); **9**, 57 (1926).
  - <sup>4</sup> Ibid., **15**, 75, 344 (1932); **16**, 74 (1933); **18**, 80 (1935).
  - <sup>5</sup> Ibid., **8**, 601 (1925); **9**, 58 (1926); **16**, 73, 298 (1933).
  - <sup>6</sup> Ibid., **7**, 91 (1923); **8**, 602 (1925); **9**, 58 (1926); **16**, 73 (1933).
  - <sup>7</sup> Ibid., **14**, 85, 416 (1931); **16**, 73, 298 (1933); **18**, 80 (1935).
  - <sup>8</sup> Ibid., **14**, 85 (1931); **16**, 74, 298 (1933); **18**, 80 (1935); **22**, 77 (1939).
  - <sup>9</sup> Ibid., **14**, 397 (1931); **16**, 74, 305 (1933); **22**, 77 (1939).
  - <sup>10</sup> Ibid., **15**, 331 (1922); **16**, 74, 293 (1933).
  - <sup>11</sup> Ibid., **10**, 50, 411 (1927); U. S. Dept. Agr. Bull. 846, p. 89.
  - <sup>12</sup> J. Assoc. Official Agr. Chem., **21**, 85 (1938).
  - <sup>13</sup> Ibid., **6**, 7 (1922); **19**, 53, 201 (1936); U. S. Dept. Agr. Bull. 846, p. 90.
-

## XXIV. FISH AND OTHER MARINE PRODUCTS

1

### APPARATUS—TENTATIVE

(a) *Funnel*.—Made of metal, tin plated or brass, 8–10" in diameter at top, with stem 3" in diameter and ca 3" long. (b) *Measures*.—Straight-sided cylindrical, made of metal, holding exactly 1 gallon and 1 quart, respectively, and having smooth rims. The plane of the rim should be level when the measure is standing on a level surface. The diameter of top of gallon measure should not be more than 5 $\frac{1}{4}$ " or less than 4 $\frac{1}{4}$ ", and that of quart measure not greater than 3 $\frac{3}{4}$ " or less than 3 $\frac{1}{4}$ ". Carefully calibrate these measures with standard glass measures. For estimating volumes short of level-full, use a graduated mechanic's depth gage to measure distance from rim to surface of contents. These depth gage readings may be tabulated against the volumes or percentage shortages as desired for each measuring vessel. (c) *Skimmer*.—A flat-bottomed pan or tray, with 2" sides. The bottom is perforated with holes  $\frac{1}{4}$ " in diameter and centers 1 $\frac{1}{4}$ " apart in a square pattern. Area of bottom of tray should be such that oysters are not over one layer deep (150 sq. in. for 1 quart of oysters). The skimmer should be supported over a solid tray slightly larger to receive the liquid. (d) *Meat chopper*.—With plate having holes  $\frac{3}{8}$ " in diameter. It should not leak around handle end. (e) *Table fork*.—With sharp-edged tines  $\frac{1}{8}$ " apart (stainless steel). (f) *Small sharp knife*. (g) *Malted milk stirrer*.—Electric.

2

### PRELIMINARY TREATMENT AND PREPARATION OF SAMPLE—TENTATIVE

To prevent loss of H<sub>2</sub>O during preparation and subsequent handling, do not use small samples. Keep the ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in following manner:

(a) *Fresh fish*.—Clean and prepare in usual manner. In the case of small fish (6" long or less), remove one longitudinal half from each of 5–10 fish. In the case of large fish, cut from each of at least 3 fish, 3 transverse slices, 1" thick: one slice from immediately back of pectoral fins, one slice halfway between first slice and vent, and one slice immediately back of vent. Separate any bones that may be present as completely as possible from pieces selected, leaving skin intact so far as possible, since in many fish large quantities of fat are stored directly beneath the skin; pass rapidly thru food chopper 3 times, thoroly mixing after each grinding; and begin all determinations as soon as practicable. If any delay occurs, chill sample to inhibit decomposition.

(b) *Canned salmon and similar types of canned fish*.—Pass entire contents of tin thru meat chopper 3 times, *thoroly mixing each time*.

(c) *Canned fish packed in oil*.—Drain fish on  $\frac{1}{4}$ " mesh sieve (or larger mesh that will retain all the meat) for 5 min. Return to sieve any meat particles passing thru. Prepare the solid portion as directed in (b). (The oil and brine may be separated and analyzed if desired.)

(d) *Shellfish other than oysters and scallops*.—If sample is received in the shell, separate edible portions in customary manner. Prepare edible portion for analysis as directed in (b).

(e) *Shell oysters and scallops*.—Wash the shells in potable H<sub>2</sub>O to remove all loose silt and dirt, and drain well. Shuck into a clean dry container enough oysters to yield at least 1 pint of drained meats. Transfer oysters to skimmer, rinse lightly with spray of H<sub>2</sub>O to remove silt particles, and pick out pieces of shell; drain 1 min. on skimmer and remove to glass fruit jar or other suitable container. Proceed as directed below, beginning "Grind the meats . . ."



(f) *Shucked oysters*.—"Fluff" the entire contents of the commercial container, or container in which sample is received (1 gallon or less in size) by pouring into standard measuring vessel thru distance of at least 1 foot, then likewise back into container, and again into measuring vessel. Measure head space with the depth gage, and determine volume. Transfer oysters to skimmer, drain 1 min., return meats to measuring vessel, and measure head space. The loss in volume is the free liquid. If less than 10%, again mix in with the meats. If the free liquid is more than 10% of total volume, allow it to set in tall container, remove scum from top, pour off clear liquid from shell and other sediment (do not filter), and analyze separately.

Grind the meats or the mixed meats and liquid in the meat chopper, remove any muscle that is retained inside chopper, and comminute with the fork and knife. (For good checks it is important to cut these pieces into small bits.) Mix all together in a tin can of suitable size and stir with electric stirrer for 5 min., keeping entire contents of container in motion. If the impeller spins in one spot without moving entire contents, raise or lower tin container. Keep prepared samples under refrigeration between 1 and 10°.

NOTE: The malted milk stirrer cannot be used successfully with ground scallop meats; they should be mixed thoroly by stirring with a spatula.

(g) *Fish packed wet in salt and brine*.—Drain off brine and rinse off adhering salt crystals with saturated salt soln. Drain again for 2 min. and proceed as directed in (a); in the case of sardine or anchovy types of small fish, as in (b).

(h) *Dried smoked or dried salt fish*.—Cut large samples into small pieces, mix, and quarter down to ca  $\frac{1}{4}$  lb. Cut, shred, grind, or otherwise comminute the  $\frac{1}{4}$  lb. sample as finely as possible so that reasonably representative samples may be weighed for analysis after being thoroly mixed. (Duplicate or triplicate determinations may be necessary to establish uniformity of sample.)

3

## TOTAL SOLIDS—TENTATIVE

(Oysters and scallops only)

Make duplicate determinations. Weigh quickly 10 g of the meats, liquid, or mixed meats and liquid in a flat-bottomed metal dish ca 9 cm in diameter. Spread sample evenly over bottom of dish. Evaporate just to dryness on steam bath and dry for 4 hours in water oven at temp. of 98–100°. Cool in desiccator and weigh promptly.

4

## ASH—OFFICIAL, FIRST ACTION

Dry a sample representing ca 2 g of dry material and proceed as directed under XXXIV, 9 or 10, at a temp. not to exceed 550°. If material contains large quantity of fat, make preliminary ashing at sufficiently low temp. to allow smoking off of fat without burning.

## SALT (CHLORINE AS SODIUM CHLORIDE)—OFFICIAL, FIRST ACTION

I. *Open Carius Method*

5

## REAGENTS

(a) *Silver nitrate soln*.—0.1 N. Standardize against 0.1 N NaCl soln containing 5.846 g of pure dry NaCl per liter.

(b) *Ammonium thiocyanate soln*.—Standardize against the 0.1 N AgNO<sub>3</sub> soln.

(c) *Ferric indicator*.—A saturated soln of ferric ammonium alum.

6

## DETERMINATION

In the case of oysters and scallops, put 10 g of the meats, liquid, or mixed meats

and liquid into a 250 ml Erlenmeyer flask or beaker. In the case of other fish products, use a suitably sized sample, depending on salt content. Add a known volume of the  $\text{AgNO}_3$  soln, more than sufficient to precipitate all the Cl as  $\text{AgCl}$  and then add 20 ml of  $\text{HNO}_3$ . Boil gently on hot plate or sand bath until all solid matter except the  $\text{AgCl}$  is dissolved (usually 15 min.). Cool, add 50 ml of  $\text{H}_2\text{O}$  and 5 ml of indicator, and titrate excess Ag with the thiocyanate until a permanent light brown color appears. Subtract ml of 0.1  $N$  thiocyanate used from ml of 0.1  $N$   $\text{AgNO}_3$  added and calculate the quantity of Cl as  $\text{NaCl}$ . With a 10 g sample each ml of 0.1  $N$   $\text{AgNO}_3 = 0.058\%$   $\text{NaCl}$ .

7

*II. With Calcium Acetate as Fixative—Tentative*

In the case of oysters and scallops, to 10 g of ground meats or liquid in Pt dish, add and thoroly incorporate 10 ml of 10%  $\text{Ca}$  acetate soln. In the case of other fish products, use a suitably sized sample, depending on salt content. Dry on steam bath, and ash in muffle at lowest visible red heat ( $550^\circ$ ). (Complete ashing is not necessary.) Dissolve the ash in the Pt dish in 25 ml of  $\text{HNO}_3$  (1+3). Add to  $\text{HNO}_3$  soln a known volume of 0.1  $N$   $\text{AgNO}_3$  soln, more than sufficient to precipitate the chlorides as  $\text{AgCl}$ . Heat to boiling, cool, and add 5 ml of the  $\text{FeNH}_4(\text{SO}_4)_2$  indicator and titrate excess Ag with the thiocyanate until a permanent light brown color appears. From ml of 0.1  $N$   $\text{AgNO}_3$  used, calculate quantity of Cl as  $\text{NaCl}$ . With a 10 g sample, each ml of 0.1  $N$   $\text{AgNO}_3$  soln = 0.058%  $\text{NaCl}$ . Make a correction for Cl in the 10 ml of 10%  $\text{Ca}$  acetate soln if it is not free from chlorides.

8

TOTAL NITROGEN—OFFICIAL, FIRST ACTION.—See II, 21, 22, or 23.

## XXV. FLAVORING EXTRACTS

### VANILLA EXTRACT AND ITS SUBSTITUTES

#### 1 SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° with a pycnometer as directed under XIV, 3.

#### 2 ALCOHOL—OFFICIAL

Proceed as directed under XVI, 3, 4, or 5, or calculate from the sp. gr. of the distillate from the Wichmann Lead Number, 9.

#### 3 GLYCEROL—TENTATIVE

Proceed as directed under XV, 5, 6, or 7, selecting method according to quantity of sugar present. Use a quantity of the sample that contains 0.1–0.4 g of glycerol.

#### VANILLIN AND COUMARIN (GRAVIMETRIC)—OFFICIAL

(Not applicable to concentrated vanillin and coumarin preparations in which quantity of vanillin and coumarin present in 50 ml exceeds quantity dissolved by 100 ml of H<sub>2</sub>O at 20°. With such preparations use smaller quantity of sample and dilute to 50 ml.)

#### 4 PREPARATION OF SOLUTION

Measure 50 ml of the extract at 20° into 250 ml beaker bearing marks showing volumes of 80 ml and 50 ml, dilute to 80 ml, and evaporate to 50 ml in water bath kept at 70° or below. Dilute again with H<sub>2</sub>O to 80 ml and evaporate to 50 ml. Transfer to 100 ml flask, rinsing beaker with hot H<sub>2</sub>O, add 25 ml of 8% neutral Pb acetate soln, make up to mark with H<sub>2</sub>O, shake, and allow to stand 18 hours (overnight) at 37–40°. Decant into small, dry filter, reserving filtrate (Soln A) for determination of vanillin and coumarin (5), Pb number (Winton, 8) and the residual color (19).

#### 5 DETERMINATION

(a) *Vanillin*.—Transfer a 50 ml aliquot of filtrate (Soln A) to separatory funnel and extract with 4 successive 15 ml portions of ether (previously washed twice with equal volume of H<sub>2</sub>O to remove alcohol). Wash combined ether solns 4 or 5 times with NH<sub>4</sub>OH (1+11), using 10 ml the first time and 5 ml thereafter. Reserve the ether soln for determination of coumarin. Slightly acidify combined ammoniacal solns with HCl (1+2), cool, and extract in a separatory funnel with 4 portions of washed ether, using ca 40 ml altogether. Evaporate the ethereal solns at room temp., dry over H<sub>2</sub>SO<sub>4</sub>, and weigh. (The vanillin residue often appears first as oil-like droplets, which on standing crystallize into light colored masses.) If, after standing in desiccator, residue is considerably discolored or gummy, extract vanillin from it by treating with at least 15 successive portions of boiling petroleum benzin (b.p. 40° or below); combine the petroleum benzin extracts, evaporate to dryness, and weigh.

The residue, if pure vanillin, should be white crystals melting at ca 80°. Dissolve small quantity of residue in 2 drops of HCl and add a crystal of resorcin. Vanillin gives pink coloration.

(b) *Coumarin*.—Evaporate at room temp. the original ether extract obtained under (a), from which the vanillin has been removed by means of NH<sub>4</sub>OH (1+11), dry over H<sub>2</sub>SO<sub>4</sub>, and weigh.

The residue, if pure coumarin, melts at ca 67°. Dissolve a small quantity of the

residue in not more than 0.5 ml of hot  $\text{H}_2\text{O}$  and add a few drops of 0.1 N I soln. Coumarin yields a brown precipitate that finally gathers in green flecks, leaving a clear brown colored soln. The reaction is especially marked if reagent is applied with glass rod to a few drops of the soln on a white plate or tile.

#### VANILLIN (COLORIMETRIC)—OFFICIAL

6

##### REAGENT

*Phosphotungstic-phosphomolybdic acid.*—To 100 g of pure Na tungstate and 20 g of phosphomolybdic acid (free from nitrates and  $\text{NH}_4$  salts), add 100 g of sirupy  $\text{H}_3\text{PO}_4$  (containing 85%  $\text{H}_3\text{PO}_4$ ) and 700 ml of  $\text{H}_2\text{O}$ . Boil over free flame  $1\frac{1}{2}$ –2 hours, cool, filter, if necessary, and make up with  $\text{H}_2\text{O}$  to 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

7

##### DETERMINATION

Transfer to 100 ml volumetric flask a quantity of sample that contains 8–12 mg of vanillin (usually 5 ml). Add 75 ml of tap  $\text{H}_2\text{O}$  at room temp. and 4 ml of Pb soln (50 g each of basic and neutral Pb acetate per liter). Dilute to 100 ml with  $\text{H}_2\text{O}$  and mix. Filter thru dry filter paper and pipet 5 ml of clear filtrate into 50 ml volumetric flask. Into another 50 ml volumetric flask pipet 5 ml of standard vanillin soln (1 ml = 0.1 mg of vanillin). To each of these flasks add from a pipet 5 ml of the reagent, allowing it to flow down neck of flask in such a way as to wash down the vanillin soln that may be on sides of flask. Mix contents of flasks by rotating and after 5 min. dilute contents to 50 ml with saturated  $\text{Na}_2\text{CO}_3$  soln. Mix thoroly by inverting flasks several times and allow to stand at least 10 min. so that the precipitate that forms may separate completely. Filter solns thru dry filter papers and compare blue colors of clear solns in colorimeter. Report result as grams of vanillin per 100 ml of extract.

#### LEAD NUMBER—OFFICIAL

8

##### I. Winton Method<sup>3</sup>

Determine Pb as sulfate or chromate, 10(a) or 10(b), in filtrate from Pb acetate precipitate (Soln A, 4) and in filtrate from blank determination, using  $\text{H}_2\text{O}$  and 5 drops of glacial acetic acid in place of sample. Calculate Pb number and report as "Lead Number—Winton."

9

##### II. Wichmann Method<sup>4</sup>

Place 175 ml of boiled  $\text{H}_2\text{O}$  in a round-bottomed flask of 1 liter capacity. Add by means of pipet 25 ml of clear Pb acetate soln (8 g per 100 ml) and 50 ml of sample. Place flask in hole in an asbestos board that is large enough to prevent heating the upper portion of flask. (When the contents of flask are reduced to 50 ml of liquid, the level of the liquid should be even with top of the board, or slightly above it.) Connect flask to condenser, and with moderate flame distil 200 ml into volumetric flask, reserving distillate for determination of alcohol. Transfer residual soln to 100 ml volumetric flask by means of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and a bent glass rod provided with a rubber tip. When cool, dilute to 100 ml with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , mix, and filter thru dry filter (Soln A). Conduct blank determination, using 5 drops of glacial acetic acid in place of sample and distilling 150 ml instead of 200 ml. Determine Pb as directed in 10(a) or 10(b); calculate Pb number and report as "Lead Number—Wichmann."

10

## DETERMINATION OF LEAD

(a) *As sulfate*.—Pipet 10 ml of Soln A (4 or 9) into 250 ml beaker and add 25 ml of  $H_2O$ , 2 ml of  $H_2SO_4$  (1+1), and 100 ml of alcohol; stir, and allow to settle overnight. Filter on Gooch crucible, wash with alcohol, ignite at low redness, cool in desiccator, and weigh. Difference between weight of  $PbSO_4$  obtained from blank and that obtained from sample  $\times 13.66 = Pb$  number of the extract.

(b) *As chromate*.—Pipet 10 ml of Soln A (4 or 9) into 400 ml beaker and add 2 ml of glacial acetic acid, 25 ml of  $H_2O$ , and 25 ml of ca 0.1 N  $K_2Cr_2O_7$  soln. Heat beaker and contents immediately with moderate flame until precipitate changes in color from yellow to orange. Filter on Gooch crucible; wash thoroly with hot  $H_2O$  and then with a few ml each of alcohol and ether. Dry at  $100^\circ$ , cool in desiccator, and weigh. Difference between weight of  $PbCrO_4$  obtained from blank and that obtained from sample  $\times 12.82 = Pb$  number.

11

## TOTAL SOLIDS—OFFICIAL

Proceed as directed under XXXIV, 4 or 5, using 10 ml of the sample.

12

## ASH—OFFICIAL

Evaporate 10 ml of extract and proceed as directed under XXXIV, 9 or 10.

13

## ASH CONSTITUENTS.—See XII.

14

## SUCROSE—OFFICIAL.—See XXXIV, 23, 24, or 29.

## VANILLA RESINS

15

*Quantitative Method<sup>b</sup>—Tentative*

Pipet 50 ml of the extract into small beaker, add 50 ml of  $H_2O$ , and evaporate to 50 ml on steam bath. Add 50 ml of  $H_2O$  and again evaporate to 50 ml. Cool. If the mixture has an acid reaction, add 2 ml of  $HCl$  (1+1). If the mixture is not acid to litmus, add  $HCl$  (1+1), dropwise, until distinctly acid to litmus paper, than 1 ml in excess. Cover and let stand overnight. Filter, wash 6 or 7 times with ca 0.05 N  $HCl$ , (9 ml of  $HCl$  (1+1) per liter of  $H_2O$ .) Dissolve resin in warm alcohol by pouring thru filter. Evaporate alcohol in tared 50 ml beaker and dry to constant weight at  $100^\circ$ . Report results to 2 decimal places only. Reserve resin for qualitative tests.

16

*Qualitative Tests—Tentative*

Place a portion of dried residue in a few ml of 5%  $KOH$  soln. Vanilla resins dissolve, giving a deep red soln. Acidify, and a precipitate is obtained.

Dissolve a portion of the dried residue in alcohol. To a portion of the soln add a few drops of 10%  $FeCl_3$  soln; to another portion add  $HCl$ . Neither produces any marked change in color if residue consists of vanilla resins. Most other resins in alcoholic soln give color reactions with  $FeCl_3$  or  $HCl$ .

To a portion of filtrate obtained in 15, add a few drops of basic  $Pb$  acetate soln, XXXIV, 19(a). Owing to excessive quantity of organic acids, gums, and other extractive matter, the precipitate is so bulky as almost to solidify. The filtrate from this precipitate should be almost colorless.

Test another portion of the filtrate from the resin for tannin with a soln of gelatin. Tannin is present in varying but small quantities, but should not be present in excessive quantities.

17

## METHYL ALCOHOL—OFFICIAL

Proceed as directed under XVI, 23, 26, or 28, using distillate from determination of alcohol, 2.

18

## COLOR VALUE—TENTATIVE

Pipet 2 ml of the extract into 50 ml volumetric flask and dilute to mark with a mixture of equal parts of alcohol and H<sub>2</sub>O. Determine color value of this diluted extract in terms of red and yellow by means of Lovibond tintometer, using a 1" cell. To obtain color value of original extract multiply the figures for each color by 25.

19 RESIDUAL COLOR AFTER PRECIPITATION WITH LEAD ACETATE<sup>6</sup>—TENTATIVE

Determine color value, in terms of red and yellow, of filtrate from the Pb acetate precipitate obtained under 4, using 1" Lovibond cell. Multiply reading by 2 to reduce results to basis of original extract. If the actual reading of soln is greater than 5 red and 15 yellow, as may be the case if extract is highly colored with caramel, use a half or quarter inch cell, and multiply readings, respectively, by 4 or 8. To obtain percentages of the two colors remaining in the Pb acetate filtrate, divide figures for red and yellow, respectively, by corresponding figures of original extract obtained under 18 and multiply quotients by 100. Calculate also ratio of red to yellow in both extract and Pb acetate filtrate.

20

## COLORS INSOLUBLE IN AMYL ALCOHOL—TENTATIVE

Proceed as directed under XVI, 34, using 25 ml of the extract and shaking with 25 ml of the Marsh reagent instead of 20 ml.

21

## COLORING MATTERS OTHER THAN CARAMEL—TENTATIVE.—See XXI.

## LEMON AND ORANGE EXTRACTS

22

## SPECIFIC GRAVITY—OFFICIAL

Determine at 20/20° with a pycnometer, as directed under XIV, 3.

## ALCOHOL

23

*Method I.—Official*

Pipet 50 ml of the extract into 200 ml volumetric flask, noting temp.; dilute with H<sub>2</sub>O to ca 200 ml; and allow mixture to stand until oil separates in clear layer at top, or centrifuge and add H<sub>2</sub>O to bring lower meniscus of the oil to mark. Pour mixture into dry Erlenmeyer flask containing 5 g of light MgCO<sub>3</sub>, stopper, shake well, and filter quickly thru large, dry, folded filter. Introduce a 100 ml aliquot of the filtrate, measured at same temp., into 300–500 ml distillation flask, and add 50 ml of H<sub>2</sub>O. Attach flask to condenser and distil almost 100 ml. Add H<sub>2</sub>O to complete volume of distillate to 100 ml at same temp., mix well, and determine sp. gr. at convenient temp. Ascertain corresponding percentage of alcohol by volume from XLIII, Table 19, and multiply result thus obtained by 4 to obtain percentage of alcohol by volume in original sample.

24

*Method II.<sup>7</sup>—Official*

(Applicable to extracts consisting only of oil, alcohol, and water.)

Let *S* represent sp. gr. of the extract at 20/20°, as determined under 22; *O*,

sp. gr. of the oil; and  $p$ , percentage of oil found. Then  $100 - p$  = percentage of water-alcohol soln, the sp. gr. of which, represented by  $P$ , is calculated as follows:

$$S = \frac{Op + P(100 - p)}{100}, \text{ whence } P = \frac{100S - Op}{100 - p}.$$

The value of  $E$ , the alcohol equivalent of  $P$ , is obtained from XLIII, Table 19. It gives percentage of alcohol in the alcohol-water soln. To find percentage of alcohol in the extract, apply following formula:

$$\text{Percentage by volume of alcohol in extract} = E \left( 1 - \frac{p}{100} \right).$$

The value of  $O$  for lemon oil may be taken as 0.86 and for orange oil as 0.85.

25

## GLYCEROL—TENTATIVE

Proceed as directed under XV, 5, 6, or 7, selecting the method according to quantity of sugar present. Use a quantity of sample that contains 0.1–0.4 g of glycerol.

## OILS OF LEMON AND ORANGE IN EXTRACTS

26

## I. By Polarization—Official

Without diluting, polarize the extract at 20° in 200 mm tube. Divide reading in degrees Ventzke by 3.2 in the case of lemon extract and by 5.2 in the case of orange extract; in the absence of other optically active substances, the result will be the percentage of oil by volume. If cane sugar is present, determine as directed under 34 and correct reading accordingly. To obtain percentage of oil by weight from percentage by volume, multiply volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide results by sp. gr. of original extract.

27

## II. By Precipitation—Official

Pipet 20 ml of the extract into Babcock milk bottle. Add 1 ml of HCl (1+1), then 25–28 ml of H<sub>2</sub>O previously warmed to 60°. Mix, and let stand in H<sub>2</sub>O at 60° for 5 min. Centrifuge for 5 min., fill bottle with warm H<sub>2</sub>O to bring oil into graduated neck of flask, again centrifuge for 2 min., and place flask in H<sub>2</sub>O at 60° for a few minutes. Note percentage of oil by volume. If oil is present in amounts over 2%, add 0.4% to percentage of oil noted to correct for solubility of the oil. If less than 2% and more than 1% is present, add 0.3% for this correction. To obtain percentage of oil by weight from percentage by volume, multiply volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide result by sp. gr. of original extract.

## TOTAL ALDEHYDES—OFFICIAL

28

## REAGENTS

(a) *Aldehyde-free alcohol*.—Allow alcohol, containing 5 g of metaphenylenediamine hydrochloride per liter, to stand at least 24 hours with frequent shaking. (Nothing is gained by previous treatment with KOH.) Boil under reflux condenser at least 8 hours, longer if necessary; allow to stand overnight, and distil, rejecting first 10 and last 5 ml that come over. Store in dark, cool place in well-filled bottles; 25 ml of this alcohol, on standing 20 min. at 14–16° with 20 ml of the sulfite-fuchsin soln, should develop only faint pink coloration. If a stronger color is developed, repeat treatment with metaphenylenediamine hydrochloride as above.

(b) *Fuchsin-sulfite soln.*—Dissolve 0.5 g of fuchsin in 250 ml of  $H_2O$ , add an aqueous soln of  $SO_2$  containing 16 g of the gas, allow to stand until colorless or nearly so, and make up to 1 liter with  $H_2O$ . Let stand 12 hours before using and keep in refrigerator. This soln is liable to deteriorate and should be reasonably fresh when used.

(c) *Standard citral soln.*—Weigh 0.5 g of citral into 50 ml volumetric flask, make up to mark with the aldehyde-free alcohol at room temp., stopper flask, and mix by shaking. Dilute 10 ml of this soln with the aldehyde-free alcohol to 100 ml in a volumetric flask, stopper flask, and mix by shaking. 1 ml of the dilute soln = 1 mg of citral.

29

## DETERMINATION

Weigh ca 25 g of the extract in stoppered weighing flask, transfer to 50 ml volumetric flask, and dilute to mark at room temp. with aldehyde-free alcohol. Measure, at room temp., 2 ml (or other suitable quantity) of this soln into comparison tube. Add 25 ml of the aldehyde-free alcohol (previously cooled to  $14-16^\circ$ ), then 20 ml of the sulfite-fuchsin soln (also cooled), and finally make up to 50 ml mark with the aldehyde-free alcohol. Mix thoroly, stopper, and keep at  $14-16^\circ$  for 15 min. Prepare standard for comparison at same time and in same manner, using 2 ml of the standard citral soln, and compare colors developed. Calculate amount of citral present and repeat determination, using quantity sufficient to give sample approximately the strength of the standard. From this result calculate quantity of citral in the sample. If the comparisons are made in Nessler tubes, standards containing 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg of citral may be prepared and the trial comparison made against these, the final comparison being made with standards lying between 1.5 and 2.5 mg with 0.25 mg increments.

It is absolutely essential to keep the reagents and comparison tubes at required temp.,  $14-16^\circ$ . If comparisons are made in a bath (possible only when bath is of glass), discard standards within 25 min. after adding the sulfite-fuchsin soln. Give samples and standards identical treatment.

CITRAL<sup>2</sup>—OFFICIAL

(Lemon and orange extracts.)

30

## REAGENT

*Metaphenylenediamine hydrochloride-oxalic acid soln.*—Dissolve 1 g of metaphenylenediamine hydrochloride in ca 45 ml of 85% alcohol, and 1 g of crystallized oxalic acid in similar quantity of alcohol of same strength, and pour the two solns into 100 ml volumetric flask. Add 2 or 3 g of fullers' earth, dilute to mark with 85% alcohol, mix, and filter thru double folded filter.

31

## DETERMINATION

Weigh 25 g of the extract into 50 ml volumetric flask, dilute to mark with alcohol (95% by volume for extracts made with the oils; 50–95% by volume for terpeneless extracts) and mix. Pipet 2 ml or other suitable quantity of this soln into colorimeter tube, add 10 ml of the reagent, dilute to suitable volume, and compare resulting color with the colors of a set of standards containing known quantities of standard citral soln, 28(c).

32

## TOTAL SOLIDS—OFFICIAL

Proceed as directed under XVI, 6, using 10 ml of the sample measured at  $20^\circ$ .



33

## ASH—OFFICIAL

Ignite the residue from 10 ml of the extract as directed under XXXIV, 9, or 10.

34

## SUCROSE—OFFICIAL

Neutralize normal weight of the extract, evaporate to dryness, wash several times with ether, dissolve in  $H_2O$ , and proceed as directed under XXXIV, 23, 24, or 29.

35

## METHYL ALCOHOL—OFFICIAL

Proceed as directed under XVI, 26, using the distillate from determination of alcohol, 23.

36

## COLORING MATTERS—TENTATIVE.—See XXI.

37

## LEMON AND ORANGE PEEL COLOR—TENTATIVE

Place a few ml of the extract in each of 2 test tubes; to one, add slowly 3–4 volumes of  $HCl$  and to other, several drops of  $NH_4OH$ . If color is due to lemon or orange peel only, it is materially deepened by each treatment.

## LEMON AND ORANGE OILS

38

## SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at  $20/20^\circ$  with a pycnometer. See XIV, 3.

39

## INDEX OF REFRACTION—OFFICIAL

Use any standard instrument, making the reading at  $20^\circ$ . See XXXI, 8.

40

## OPTICAL ROTATION—OFFICIAL

Determine rotation at  $20^\circ$  with any standard instrument, 50 mm tube, and Na light. State results in angular degrees on 100 mm basis. If instruments having the sugar scale are used, the reading for orange oils is above the range of the scale, but readings may be obtained by the use of standard laevorotatory quartz plates, or by 25 mm tube. The true rotation cannot be obtained by diluting the oil with alcohol and correcting the rotation in proportion to the dilution.

41

TOTAL ALDEHYDES<sup>10</sup>—OFFICIAL*Fuchsin-Bisulfite Method*

Weigh a small quantity of the sample into small stoppered flask and dilute with aldehyde-free alcohol in proportion of 2 g of lemon oil or 4 g of orange oil to 10 ml of soln. Determine total aldehydes as directed under 29, expressing result as citral.

*Kleber Method<sup>11</sup>*

42

## REAGENT

*Phenylhydrazine soln.*—Prepare a 10% soln in absolute alcohol. Sufficiently pure phenylhydrazine can be obtained by distilling the commercial product in vacuo, rejecting the first portions coming over that contain  $NH_3$ .

43

## DETERMINATION

Weigh accurately ca 15 g of the sample into small, glass-stoppered flask, and add 10 ml of the phenylhydrazine soln. Allow to stand 30 min. at room temp. and titrate

with 0.5 *N* HCl, using either methyl or ethyl orange indicator. Titrate similarly 10 ml of the phenylhydrazine soln. Difference in number of ml of 0.5 *N* acid used in these 2 titrations  $\times$  factor 0.076 = weight of citral in sample. If difficulty is experienced in detecting end point of the reaction, titrate until soln is distinctly acid; transfer to separatory funnel and draw off alcoholic portion. Wash the oil with H<sub>2</sub>O, adding washings to alcoholic soln, titrate back with 0.5 *N* alkali, and make necessary corrections.

44

*Hiltner Method*<sup>11</sup>

Weigh accurately ca 2 g of lemon oil or 8 g of orange oil into 100 ml volumetric flask, dilute to mark with alcohol, and proceed as directed under 31, using 2 ml of the dilute soln for the comparison.

45

PHYSICAL CONSTANTS OF THE 10 PER CENT DISTILLATE<sup>12</sup>—OFFICIAL

Place 50 ml of the sample in 3-bulb Ladenburg flask having main bulb 6 cm in diameter and of 120 ml capacity and the condensing bulbs of following dimensions: 3.5 cm, 3 cm, and 2.5 cm. The distance from bottom of flask to opening of side arm should be 20 cm. Distil the oil at rate of 2 ml per min. until 5 ml has been distilled. Determine refractive index and rotation of this distillate as directed under 39 and 40.

46

PINENE<sup>13</sup>—OFFICIAL

Mix the 10% distillate, 45, with 5 ml of glacial acetic acid, cool mixture thoroly in freezing bath, and add 10 ml of ethyl nitrite. Add slowly, with constant stirring, 2 ml of HCl (2+1). Keep mixture in freezing bath 15 min. Collect crystals formed on filter, using suction, and wash with alcohol. Return combined filtrate and washings to freezing bath for 15 min. Collect additional crystals formed on original filter. Wash combined crops of crystals thoroly with alcohol. Dry at room temp. and dissolve in minimum quantity of CHCl<sub>3</sub>. Add methyl alcohol to the CHCl<sub>3</sub> soln, a little at a time, until the nitroso-chlorides crystallize out. Mount the separated and dried crystals in olive oil and examine under microscope. Pinene nitroso-chloride crystals have irregular pyramidal ends, while limonene nitroso-chloride crystallizes in needles.

## ALMOND EXTRACT

47

## ALCOHOL—TENTATIVE

As almond extract usually contains only ca 1% of almond oil, in most cases the alcohol can be calculated from the sp. gr. of the extract. If the extract is high in solids, proceed as follows: Add 25 ml of the extract, measured at room temp., to 75 ml of saturated NaCl soln in separatory funnel, and extract twice with 50 ml portions of petroleum benzin (b.p. 40–60°). Collect the petroleum benzin extract in second separatory funnel and wash twice with 2 portions (25 ml) of saturated brine. Combine original salt soln with the washings, add a little powdered pumice, and distil into 100 ml volumetric flask. When almost 100 ml has been distilled, make up to mark with H<sub>2</sub>O at room temp. and determine alcohol from the sp. gr. as directed under XIV, 3, using Table 19, XLIII.

48

## BENZALDEHYDE—TENTATIVE

Measure out 2 portions of 10 ml each of the extract into 300 ml Erlenmeyer flasks and add 10 ml of phenylhydrazine soln (3 ml of glacial acetic acid, 40 ml of H<sub>2</sub>O, 2 ml of phenylhydrazine) to one flask and 15 ml to the other. Allow mixtures to

stand overnight in dark place. Add 200 ml of  $H_2O$ , and filter thru weighed Gooch crucible provided with thin layer of asbestos. Wash precipitate first with cold  $H_2O$  and finally with 10 ml of 10% alcohol. Dry at  $70^\circ$  for 3 hours at pressure not to exceed 100 mm of Hg or to constant weight over  $H_2SO_4$ . Weight of precipitate  $\times$  factor 5.408 = weight of benzaldehyde in 100 ml of sample. If duplicate determinations do not agree, repeat operation, using a larger quantity of the phenylhydrazine soln.

49

BENZOIC ACID<sup>14</sup>—TENTATIVE

Measure 10 ml of the extract into 100 ml flask and add 10 ml of 10% NaOH soln and 20 ml of 3%  $H_2O_2$  soln; cover with watch-glass and place in water oven. Oxidation of the aldehyde to benzoic acid begins almost immediately and should continue 5–10 min. after all odor of benzaldehyde has disappeared (20–30 min.). Remove flask from water oven; transfer contents to separatory funnel, rinsing off watch-glass; add 10 ml of  $H_2SO_4$  (1+5); and cool contents of funnel to room temp. under water tap. Extract the benzoic acid with 4 portions of 25, 25, 20, and 20 ml of ether, respectively, and wash combined extracts with 2 portions of 5–10 ml of  $H_2O$ , or until all  $H_2SO_4$  is removed. Filter into weighed dish, evaporate at room temp., dry overnight in desiccator, and weigh the benzoic acid. Multiply result by 10.

Multiply grams per 100 ml of benzaldehyde obtained under 48 by 1.151 to obtain equivalent of benzoic acid and subtract this product from grams per 100 ml of total benzoic acid obtained above. Difference = grams of benzoic acid per 100 ml of extract.

## HYDROCYANIC ACID

50

*Qualitative Test—Tentative*

Add several drops of freshly prepared 3%  $FeSO_4 \cdot 7H_2O$  soln and a single drop of 1%  $FeCl_3 \cdot 6H_2O$  soln to several ml of the extract. Mix thoroly and add 10% NaOH soln, dropwise, until no further precipitate forms and then  $H_2SO_4$  (1+9) to dissolve the precipitate. In presence of even small quantities of HCN, a Prussian blue coloration or suspension will develop.

51

*Quantitative Method—Tentative*

(In absence of chlorides.)

Measure 25 ml of the extract into small flask and add 5 ml of freshly precipitated  $Mg(OH)_2$ , Cl-free. Titrate with 0.1 N  $AgNO_3$  soln, using  $K_2CrO_4$  as an indicator. 1 ml of 0.1 N  $AgNO_3$  = 0.0027 g of HCN.

## NITROBENZENE

52

*Qualitative Test—Tentative*

Boil a few ml of the extract with some Zn dust and acetic acid and filter. Add to filtrate a drop of  $CHCl_3$ , make strongly alkaline with 10% NaOH soln, and heat. Presence of nitrobenzene in the original extract is indicated by development of characteristic odor of phenylisocyanide.

## CASSIA, CINNAMON, AND CLOVE EXTRACTS

53

## ALCOHOL—TENTATIVE.—See 47.

OIL<sup>14</sup>—TENTATIVE

54

*Method I*

Transfer 10 ml of the extract to separatory funnel, add 30 ml of  $H_2O$ , acidify with 1 ml of HCl (1+1), and extract 3 times with ether, using not less than 100 ml alto-

gether. Wash combined ether solns twice with  $H_2O$ , and in the case of cinnamon extract dry by shaking with small quantity of granulated  $CaCl_2$ . Transfer to weighed wide-mouthed weighing bottle and evaporate the ether as rapidly as possible on boiling water bath, rotating liquid onto sides of bottle to rid residual oil of traces of ether. Weigh residue and divide weight by sp. gr. of the oil in order to obtain percentage of oil by volume. In the case of clove oil, allow weighing bottle to remain in balance case until usual film of moisture has evaporated. The time of weighing, however, should not be delayed over 3 min. Determine refractive index of residual oils at  $20^\circ$ . Dissolve a drop of the oil in several drops of alcohol and add a drop of 10%  $FeCl_3 \cdot 6H_2O$ .

*Specific gravity, refractive index at  $20^\circ$ , and color reaction with  $FeCl_3$  soln*

OIL	SPECIFIC GRAVITY	REFRACTIVE INDEX AT $20^\circ$	COLOR REACTION WITH $FeCl_3$ SOLN
Cassia.....	1.05	1.585-1.600	Brown
Cinnamon.....	1.03	1.590-1.599	Green
Cloves.....	1.055	1.560-1.565	Deep blue

55

*Method II<sup>15</sup>*

(Applicable to extracts of cinnamon and clove.)

Pipet 10 ml of the extract into standard Babcock milk bottle. Remove nearly all alcohol by blowing air into bottle thru small glass tube 30 min., or longer if necessary. Add from 10 ml buret 1 ml of solvent (equal parts of U.S.P. mineral oil and  $H_2O$ -free kerosene), shake well, and fill with a saturated soln of  $MgSO_4$ . Centrifuge 10 min. and read volume of oil from extreme bottom to extreme top of column. To obtain percentage of oil subtract 5 divisions and multiply remainder by 2.

**GINGER EXTRACT**

56

ALCOHOL—TENTATIVE.—See XVI, 3, 4, or 5.

57

SOLIDS—TENTATIVE

Evaporate 10 ml of the extract nearly to dryness on steam bath, dry 2 hours in water oven at temp. of boiling  $H_2O$  and weigh.

58

GINGER (QUALITATIVE TEST)—TENTATIVE

Dilute 10 ml of the extract to 30 ml, evaporate to 20 ml, decant into separatory funnel, and extract with equal volume of ether. Allow ether to evaporate spontaneously in porcelain dish, and to residue add 5 ml of 75%  $H_2SO_4$  and ca 5 mg of vanillin. Allow to stand 15 min. and add an equal volume of  $H_2O$ . In the presence of ginger extract an azure blue color develops.

59

CAPSICUM (QUALITATIVE TEST)—TENTATIVE

To 10 ml of the extract add cautiously  $NaOH$  soln (1+9) until soln reacts very slightly alkaline with litmus paper. Evaporate at ca  $70^\circ$  to ca  $\frac{1}{4}$  original volume and render slightly acid with  $H_2SO_4$  (1+9), testing with litmus paper. Transfer to separatory funnel, rinsing dish with  $H_2O$ , and extract with equal volume of ether, avoiding formation of emulsion by shaking funnel gently 1-2 min. Draw off lower layer and wash ether extract once with ca 10 ml of  $H_2O$ . Transfer washed ether extract to small evaporating dish, render decidedly alkaline with 0.5 *N* alcoholic  $KOH$ , and evaporate at ca  $70^\circ$  until residue is pasty. Add ca 20 ml more of the 0.5 *N* alcoholic  $KOH$  and allow mixture to stand on steam bath until gingerol is com-

pletely saponified (ca 30 min.). Dissolve residue in a little  $H_2O$  and transfer with  $H_2O$  to small separatory funnel. The volume should not exceed 50 ml. Extract alkaline soln with equal volume of ether. Wash ether extract repeatedly with small quantities of  $H_2O$  until no longer alkaline to litmus. Transfer washed extract to small evaporating dish and allow ether to evaporate spontaneously. Finally test residue for capsicum by moistening tip of the finger, rubbing it on bottom and sides of dish, and then applying finger to end of tongue. A hot, stinging, or prickly sensation, which persists for several minutes, indicates capsicum or other foreign pungent substances.

### PEPPERMINT, SPEARMINT, AND WINTERGREEN EXTRACTS

60

ALCOHOL—TENTATIVE.—See 47.

61

OIL<sup>16</sup>—TENTATIVE

Pipet 10 ml of the extract into Babcock milk bottle, add 1 ml of  $CS_2$ , mix thoroly, and add 25 ml of cold  $H_2O$  and 1 ml of  $HCl$ . Close mouth of bottle and shake vigorously; centrifuge 6 min., and remove all but 3–4 ml of supernatant liquid, which should be practically clear, by aspirating thru glass tube of small bore. Connect the stem of the bottle with filter pump and immerse bottle in  $H_2O$  kept at ca  $70^\circ$  for 3 min., removing from bath every 15 seconds and shaking vigorously. Continue in same manner for 45 seconds, using boiling water bath. Remove from bath and shake while cooling. Disconnect from the suction and fill the bottle to neck with saturated  $NaCl$  soln at room temp., centrifuge for 2 min., and read volume of separated oil from top of meniscus. Multiply reading by 2 to obtain percentage of oil by volume. In the case of wintergreen, use as floating medium a mixture of 1 volume of  $H_2SO_4$  and 3 of saturated  $Na_2SO_4$  soln.

62

METHYL SALICYLATE IN WINTERGREEN EXTRACT<sup>14</sup>—TENTATIVE

Mix 10 ml of the extract with 10 ml of 10%  $KOH$  soln. Heat on steam bath until volume is reduced ca one-half. Add distinct excess of  $HCl$  (1+1), cool, and extract with 3 portions of ether, 40, 30, and 20 ml, respectively. Filter extract thru dry filter into weighed dish, wash paper with 10 ml of ether, and allow filtrate and washings to evaporate spontaneously. Dry in desiccator containing  $H_2SO_4$  and weigh. Weight of salicylic acid so found  $\times 9.33$  = percentage by volume of methyl salicylate in sample.

### ANISE AND NUTMEG EXTRACTS

63

OIL<sup>14</sup>—TENTATIVE

To 10 ml of the extract in Babcock milk bottle, add 1 ml of  $HCl$  (1+1), then sufficient half-saturated salt soln, previously heated to  $60^\circ$ , to fill flask nearly to neck. Cork and let stand in  $H_2O$  at  $60^\circ$  ca 15 min., rotate occasionally, and centrifuge 10 min. at ca 800 r.p.m. Fill bottle to neck with saturated  $NaCl$  soln and again centrifuge 10 min. If separation is not satisfactory or liquid is not clear, cool to ca  $10^\circ$  and centrifuge for an additional 10 min. Reading  $\times 2$  = percentage of oil by volume.

64

ESSENTIAL OIL IN EXTRACTS AND TOILET PREPARATIONS<sup>17</sup>

(Applicable to extracts of allspice, anise, caraway, lemon, nutmeg, orange, peppermint, pimiento, rosemary, thyme, wintergreen, and methyl salicylate.)

Pipet 10 ml of sample (5 ml when oil content exceeds 5% by volume) into standard Babcock milk bottle, add 0.50 ml of solvent (equal parts of U.S.P. mineral oil

and H<sub>2</sub>O-free kerosene) and 1 ml of HCl (1+1), and fill to shoulder with saturated NaCl soln. Shake bottle 3 min., then add the salt soln to bring column of oil within graduations on neck. Centrifuge 10 min. at high speed and read volume of oil from extreme bottom to extreme top of column. (Read from extreme bottom to bottom of meniscus at top of column for allspice, peppermint, and pimiento extracts.) To obtain percentage of oil subtract 2.5 divisions and multiply remainder by 2. (Multiply by 4 if 5 ml sample is used.)

#### LEMON, ORANGE, OR LIME OIL IN VEGETABLE AND MINERAL OILS

##### I. By Steam Distillation<sup>18</sup>—Official

65

###### APPARATUS

(a) *Steam generator filled with H<sub>2</sub>O*.—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask*.—A Kjeldahl flask of ca 750 ml capacity, with shortened neck, ca 10" in height over all.

(c) *Spray tube*.—A glass tube with small perforated bulb at end passing thru rubber stopper and reaching to bottom of distillation flask.

(d) *Bent glass tube*.—About 8 mm in diameter. Connects distillation flask to upright condenser. The shape of this tube allows vapor condensing in tube to return to distillation flask.

(e) *Liebig condenser*.—With 20" water jacket.

(f) *Wilson receiving flask*.—Shaped like Babcock test bottle with graduated neck but of much larger capacity and with vertical glass outlet tube sealed on near bottom. Upper end of outlet tube is turned down. Capacity of flask is ca 250 ml. The neck may consist of portion of buret graduated from 0–25 ml with top flared out. The outlet tube is ca 3 mm in diameter, and end is at such height that when flask is filled with H<sub>2</sub>O the meniscus in neck will be between 0 and 1 ml marks.

66

###### DETERMINATION

Measure 100 ml of the sample in graduated cylinder and transfer to distillation flask. Immerse flask in water bath and connect with condenser by means of the bent glass tube. Fill receiving flask with H<sub>2</sub>O and so place under condenser that end of condenser will be ca 0.5" above level of H<sub>2</sub>O in receiving flask. Place 200 ml graduated cylinder under end of outlet tube to catch displaced liquid. Heat water bath to boiling and pass steam thru sample until 200 ml of liquid has been collected in graduated cylinder.

Disconnect apparatus, allow receiving flask to stand 15 min., or until separation of oil is complete, and read volume of oil obtained. Calculate percentage (by volume) of essential oil in sample by dividing reading by 0.90 for lemon oil in corn and cottonseed oils, 0.95 for orange oil in corn and cottonseed oils, and by 0.78 for distilled or expressed lime oil in corn and cottonseed oils. Where menstruum is mineral oil, subtract 0.3 ml from reading before dividing by factors 0.90, 0.95, and 0.78 for lemon oil, orange oil, and lime oil, respectively.

67

##### II. By Polarization<sup>19</sup>—Tentative

Polarize the sample at 20° in 200 mm tube, making 5 readings. From average of these readings in degrees Ventzke subtract, for corn oil +0.6°, for cottonseed oil –0.3°, for peanut oil +0.2°, and for mineral oil +5.5°, as correction for rotatory effect of menstruum. To obtain percentage by volume of essential oil in mixture, divide corrected polariscopic reading so obtained by factor 3.4 for lemon oil in corn

oil, 3.7 for lemon oil in cottonseed oil, 3.6 for lemon oil in peanut oil, 3.5 for lemon oil in mineral oil, 5.4 for orange oil in corn oil, 5.7 for orange oil in cottonseed oil, 5.6 for orange oil in mineral oil, 2.0 for lime oil in corn oil, 2.3 for lime oil in cottonseed oil, and 2.2 for lime oil in mineral oil.

68

 **$\beta$ -IONONE—OFFICIAL, FIRST ACTION**

(Applicable to pure solutions of 100 mg or less in 5 ml of alcohol.)

Place 5 ml of alcohol containing 10–100 mg of  $\beta$ -ionone in 125 ml conical flask. Add 95–100 mg of solid *m*-nitrobenzhydrazide and dissolve by warming soln on steam bath, taking precautions to prevent loss of alcohol thru evaporation. Add 5 ml of H<sub>2</sub>O, and if soln becomes cloudy, warm until clear. Remove soln from steam bath, add 0.2 ml of glacial acetic acid, stopper flask lightly, and place upon a wooden surface to prevent too rapid cooling. If ca 20 mg or more of  $\beta$ -ionone is present, crystals will begin to form within 30 min. after contents of flask have reached room temp. Let stand in room at least 2 hours (overnight does no harm) and add 5 ml of H<sub>2</sub>O dropwise, mixing soln continuously during the addition by rotating flask. Stopper, let stand in room at least 1 hour, and place in refrigerator overnight (not longer than 48 hours). Filter thru No. 3 or 4 sintered glass crucible, wash with 30 ml of dilute alcohol (3+7), using a wet policeman to remove precipitate adhering to flask, and dry at 100°. Weight of precipitate  $\times 0.541$  = corresponding weight of  $\beta$ -ionone. Identify crystals microscopically, 71.

 **$\beta$ -IONONE IN RASPBERRY FLAVORS**

69

**APPARATUS**

- (a) *Steam generator filled with water.*—Oil can holding 1 gallon will serve purpose.
- (b) *Distillation flask.*—Round-bottomed boiling flask having interchangeable ground-glass connection 24/40, capacity about twice the volume of sample to be used.
- (c) *Still head.*—Adapter, 75° angle, with interchangeable male connections 24/40 at bottom and side and female connection 14/35 at top, with side arm lengthened and bent to fit vertical condenser.
- (d) *Spray tube.*—Adapter, for use with Woulff bottles equipped with interchangeable ground-glass connection, aeration tube with connection 14/35, holes in bulb ca 2 mm in diameter, length of tubing such that when apparatus is set up, the bulb is situated not more than 20 mm above bottom of distilling flask.
- (e) *Condenser.*—Coil type with interchangeable female connection 24/40 at top with 250 to 300 mm jacket and outlet tube lengthened to ca 200 mm to reach bottom of receiving flask.
- (f) *Receiving flask.*—Conical flask of 500 ml capacity.

70

**DETERMINATION**

Place 250–1000 ml of sample (should contain not more than 100 mg of  $\beta$ -ionone) in distilling flask and connect with apparatus. Add enough H<sub>2</sub>O to receiving flask to just cover outlet of condenser. Heat sample nearly to boiling on asbestos mat with flame or by immersing it in boiling water bath. As soon as sample has reached temp. of bath or has just begun to boil, connect with steam generator and pass rapid current of steam thru sample until ca 500 ml of distillate has been collected.

Add sufficient H<sub>2</sub>O to distillate to reduce the alcohol content to ca 10% or less and transfer to large separatory funnel. Add 150–200 ml of ether, depending upon volume of soln, so that ca 100 ml will be obtained upon separation.

Shake thoroly ca 2 min. After allowing mixture to settle till clear, draw off watery soln till ca 25 ml remains in separator. Whirl liquid and again allow to settle. When clear, draw off remainder of watery soln, then draw off ether soln into 125 ml conical flask containing 95–100 mg of *m*-nitrobenzhydrazide. After separator has drained ca 1 min., close stopcock, pour 10–15 ml of ether into separator to wash down sides, and allow soln to settle 1 min., then add to main ether soln. Add 0.2 ml of acetic acid and dissolve the solid reagent by stirring and breaking up lumps with glass rod, warming if necessary to complete soln. Permit mixture to stand ca 1 hour and evaporate on steam bath to ca 10 ml, passing current of air into flask to hasten evaporation and keep down temp. In meantime make second extraction of distillate, using 100 ml of ether. Add the separated ether soln to flask containing residue from first ether extract, follow with ether washings of separator, and after allowing to stand at least 15 min. evaporate to 10 ml as before. Similarly make third extraction, using 100 ml of ether, add to flask, and evaporate as before until 1–3 ml of watery liquid and perhaps some oily residue remain.

While flask is still warm, add 5 ml of alcohol from pipet, allowing liquid to wash down sides of flask, and dissolve residue completely by warming on steam bath, protecting liquid against loss by evaporation. Add 5 ml of H<sub>2</sub>O and warm if necessary to obtain a clear soln. Add 0.2 ml of acetic acid, close with cork stopper, and place flask on a wooden surface to prevent too rapid cooling.

After 2 hours add 5 ml of H<sub>2</sub>O dropwise, mixing liquid by continuously rotating flask, stopper, and keep at room temp. at least 1 hour (overnight does no harm), then place in refrigerator overnight (not longer than 48 hours).

Filter on fritted glass crucible of porosity 3 or 4 and wash with ca 30 ml of dilute alcohol (3+7). Dry in vacuum oven at 70° and weigh. Weight of precipitate  $\times 0.541 = \beta$ -ionone. Identify crystals microscopically, 71.

If precipitated material consists of oily matter mixed with crystalline matter, place fritted glass crucible in Gooch holder attached to suction flask. By means of wire, support a test tube within the suction flask in such manner as to catch any liquid that may pass thru crucible. Add ca 5 ml of petroleum benzin, cover crucible, and let stand ca 5 min. Turn on suction just long enough to carry thru any solvent that may remain in crucible. Transfer petroleum benzin soln to small beaker and allow to evaporate spontaneously. Repeat several times until no more soluble matter is obtained by the extraction. Examine remaining contents of crucible and the several residues microscopically for crystals of  $\beta$ -ionone-*m*-nitrobenzhydrazide.

71

OPTICAL PROPERTIES OF  $\beta$ -IONONE-*m*-NITROBENZHYDRAZIDE

To the naked eye this substance in mass has a yellowish color, but when examined in ordinary light under microscope, it is essentially colorless and crystallizes in thin, rod-like plates, many having lath-like or frayed ends, some having six-sided outline. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative. The refractive indices are the minimum and maximum values,  $n_{\alpha} = 1.548$ , invariably shown on the elongated fragments when their long dimension is parallel to vibration plane of lower nicol (lengthwise), and  $n_{\gamma} = 1.648$ , usually shown on elongated fragments when their long dimension is at right angles to vibration plane of lower nicol (crosswise).

## SELECTED REFERENCES

- <sup>1</sup> J. Am. Chem. Soc., 21, 256 (1899); 24, 1128 (1902); 27, 719 (1905).
- <sup>2</sup> J. Assoc. Official Agr. Chem., 8, 688 (1925).
- <sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull., 132, p. 109.
- <sup>4</sup> J. Ind. Eng. Chem., 13, 414 (1921); J. Assoc. Official Agr. Chem., 8, 689, 691 (1925); 47, 87 (1926).



- <sup>5</sup> Ibid., 9, 48 (1926).  
<sup>6</sup> U. S. Dept. Agr. Bur. Chem. Bull. 152, p. 146.  
<sup>7</sup> J. Ind. Eng. Chem., 1, 84 (1909); J. Assoc. Official Agr. Chem., 4, 472 (1921);  
5, 308 (1922).  
<sup>8</sup> J. Am. Chem. Soc., 28, 1472 (1906); J. Assoc. Official Agr. Chem., 14, 68 (1931).  
<sup>9</sup> J. Assoc. Official Agr. Chem., 12, 48 (1929).  
<sup>10</sup> J. Am. Chem. Soc., 28, 1472 (1906).  
<sup>11</sup> U. S. Dept. Agr. Bur. Chem. Bull., 137, p. 72.  
<sup>12</sup> Schimmel and Co., Semi-annual Rpt., Oct. 1898, p. 41.  
<sup>13</sup> U. S. Dept. Agr. Bur. Chem. Circ. 46, p. 9.  
<sup>14</sup> J. Ind. Eng. Chem., 1, 84 (1909).  
<sup>15</sup> J. Assoc. Official Agr. Chem., 15, 539 (1932); 16, 75 (1933).  
<sup>16</sup> J. Ind. Eng. Chem., 3, 252 (1911).  
<sup>17</sup> J. Assoc. Official Agr. Chem., 15, 539 (1932); 16, 75 (1933); 19, 75 (1936).  
<sup>18</sup> Ibid., 9, 450 (1926); 11, 45, 503 (1928).  
<sup>19</sup> Ibid., 9, 453 (1926); 10, 495 (1927).  
<sup>20</sup> Ibid., 22, 383 (1939).  
<sup>21</sup> Ibid., 386, 390.

## XXVI. FRUITS AND FRUIT PRODUCTS

1

### SAMPLING—TENTATIVE

*Boxed fruit.*—Remove cover, bottom, or one side of box, as is most convenient. Remove a block comprising  $\frac{1}{8}$  of contents of box taken from one corner as follows: With sharp knife make vertical cut midway between *ends* of box to center of top surface, this cut to extend half way to the bottom. Make another vertical cut midway between *sides* of box, extending half way to the bottom, and continue it until it meets the first cut. Remove all fruit included in the angle formed by the two cuts. Working rapidly, break up all lumps, thoroly mix, and take sufficient sample to fill quart Mason jar, replacing remainder in box. Seal jar and send to laboratory. Sample sufficient number of boxes taken from different parts of pile to constitute at least the square root of the lot.

2

### PREPARATION OF SAMPLE—OFFICIAL

Without delay transfer all samples received in open packages (*i.e.*, not in sterile condition) to glass-stoppered containers and keep in cool place. Make the determinations of alcohol, total and volatile acids, solids, and sugars, particularly in the case of fruit juices and fresh fruits, at once, as fermentation is liable to begin very soon. (Portions for determination of sucrose and reducing sugars may be weighed and kept for several days without fermenting if the slight excess of neutral Pb acetate soln required in the determination is added.) Prepare the various products for analysis as follows:

(a) *Juices.*—Mix thoroly by shaking to insure uniformity in sampling and filter thru muslin previously washed and dried. Prepare fresh juices by pressing the well-pulped fruit in jelly bag and filtering thru muslin previously washed and dried. Express juice of citrus fruit by one of common devices used for squeezing oranges or lemons, and strain expressed juice thru muslin previously washed and dried.

(b) *Jellies and sirups.*—Mix thoroly to insure uniformity in sampling.

(b<sub>1</sub>) *Preparation of soln.*—Weigh 300 g of the thoroly mixed sample into 2 liter flask and dissolve in H<sub>2</sub>O, heating on steam bath, if necessary. Apply as little heat as possible to minimize inversion of sucrose. Cool, dilute to mark, mix thoroly by shaking, and use aliquots for the various determinations. If insoluble material is present, mix thoroly and filter before taking aliquots.

(c) *Fresh fruits, dried fruits, preserves, jams, and marmalades.*—Pulp by grinding in large mortar or by passing thru food chopper and mix thoroly, completing operation as quickly as possible to avoid loss of moisture. In the case of dried fruits, pass sample thru food chopper three times, mixing thoroly after each grinding. Set the burrs or blades of food chopper as close as possible without crushing seeds. If container is No. 10 can or smaller, grind entire contents. Mix contents of larger containers thoroly by stirring and remove portion for grinding. In the case of stone fruits, remove pits, and determine their proportion in weighed sample. In the case of canned fruits, an examination of the sirup in which the fruits are preserved is often sufficient. Separate liquor by draining (*cf.* XXXV, 2) and treat as directed under (a).

(c<sub>1</sub>) *Preparation of soln.*—Weigh 300 g of the well-pulped and mixed sample into a 1.5–2 liter beaker; add ca 800 ml of H<sub>2</sub>O; and boil 1 hour, replacing at intervals the H<sub>2</sub>O lost by evaporation. Transfer to 2 liter volumetric flask, cool, dilute to volume, and filter. With unsweetened fruit it is desirable, tho not actually necessary, to add sugar before boiling; therefore weigh 150 g of fruit, add 150 g of sugar and 800 ml of H<sub>2</sub>O, and proceed as directed previously.

(d) *Canned fruits*.—See XXXV, 2. Carefully invert by hand all fruits having cups or cavities if they fall on sieve with cups or cavities up. Cups or cavities in soft products may be drained by tilting the sieve, but no other handling of these products while draining is permissible.

3

## ALCOHOL—OFFICIAL

Determine alcohol in 50 g of the original material as directed under XIV, 5.

4

MOISTURE<sup>a</sup>—OFFICIAL

*Dried fruits*.—Spread 5–10 g of the prepared sample, 2(c), as evenly as possible over bottom of metal dish ca 8.5 cm in diameter and provided with a tightly fitted cover, weigh, and dry at 70° for 6 hours under pressure not to exceed 100 mm of Hg. During drying admit to oven slow current of air (ca 2 bubbles per second) dried by passing thru H<sub>2</sub>SO<sub>4</sub>. (The metal dish must be placed in direct contact with metal shelf of oven.) Replace cover, cool dish in desiccator, and weigh. Disregard any temporary drop of oven temp. that may occur during early part of drying period owing to rapid evaporation of H<sub>2</sub>O. With raisins and fruit similarly rich in sugar, use ca 5 g of sample and dry and weigh with dish ca 2 g of finely divided asbestos. Moisten with hot H<sub>2</sub>O, mix sample and asbestos thoroly, evaporate on steam bath barely to dryness, and complete drying as directed above.

## TOTAL SOLIDS—OFFICIAL

5

## I. Insoluble Matter Present

*Fresh and canned fruits, jams, marmalades and preserves*.—Weigh accurately into large flat-bottomed dish 20 g of pulped fresh fruit, or a quantity of fruit products that will give not more than 3–4 g of dry material. If necessary to secure a thin layer of the material, add a few ml of H<sub>2</sub>O and mix thoroly. Dry at 70° under pressure not to exceed 100 mm of Hg until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg.

6

## II. No Insoluble Matter Present

*Fruit juices, jellies and sirups*.—Proceed as directed under XXXIV, 4, 6, 7, or 8, using sample prepared as directed under 2(a) or (b).

7

WATER-INSOLUBLE SOLIDS<sup>a</sup>—TENTATIVE

Weigh 25 g of the prepared sample, 2(c), into 400 ml beaker; add 200 ml of H<sub>2</sub>O, cover, heat to boiling, and boil vigorously 30 min., replacing at intervals H<sub>2</sub>O lost by evaporation. As the filtering medium, use weighed piece of cotton 5" square, of a thickness about one-half that of the layer in the ordinary 16 ounce roll of absorbent cotton. Tear a piece of the cotton off one corner and use to plug neck of the funnel lightly. Then arrange the large piece in funnel, and filter sample. So pour the hot H<sub>2</sub>O that the pulp is loosened from the cotton with each addition (usually 700–800 ml of filtrate is collected). Fold cotton and contents and remove excess H<sub>2</sub>O by gently squeezing the cotton while it is still in the funnel. Dry the material to constant weight at 100°.

8

SOLUBLE SOLIDS IN FRESH AND CANNED FRUITS, JAMS, MARMALADES AND PRESERVES<sup>a</sup>—TENTATIVE

(Insoluble matter present.)

Proceed as directed under XXXIV, 8. Percentage of soluble solids = % of solids determined by refractometer  $\times \frac{100-b}{100}$ , in which b = water-insoluble solids.

9

## TOTAL ASH—OFFICIAL

Proceed as directed under XXXIV, 9 or 10, the temp. of ashing not to exceed 525°, using 25 g of juices, fresh fruits, or canned fruits, and 10 g of jellies, sirups, preserves, jams, marmalades, or dried fruits.

If the ash of the water-soluble portion only is desired, evaporate on steam bath to dryness 100 ml of the prepared soln, 2(b<sub>1</sub>) or 2(c<sub>1</sub>), and proceed as directed under XXXIV, 9 or 10.

10

## ALKALINITY OF THE ASH—OFFICIAL

Into the Pt dish containing the ash obtained under 9 introduce a measured excess of 0.1 *N* HCl, warm on steam bath, cool, add a few drops of methyl orange indicator, and titrate excess acid with 0.1 *N* NaOH soln. Report as alkalinity, number of ml of 0.1 *N* acid required to neutralize the ash from 100 g of sample, and as alkalinity number, number of ml of *N* acid required to neutralize 1 g of ash. Reserve soln for determination of S in ash.

11

## SULFUR IN ASH—OFFICIAL

(For products containing a basic ash.)

Add 5 ml of HCl (1+2.5) to the soln remaining after determination of alkalinity of ash, 10, and evaporate to dryness. Heat to 110° for 1 hour to dehydrate any SiO<sub>2</sub>. Take up in 5 ml of the dilute HCl and filter, washing filter paper well with hot H<sub>2</sub>O. Heat filtrate to boiling and add dropwise from buret or pipet 5 ml of 10% BaCl<sub>2</sub> soln. Evaporate to 100 ml and let stand overnight. Filter on weighed Gooch or Munroe crucible or on 7 cm ashless filter paper, wash with hot H<sub>2</sub>O until filtrate is free from chlorides, dry, ignite over Bunsen burner, and weigh as BaSO<sub>4</sub>. As quantity of precipitate is small, exercise great care and make determination in duplicate. Report result as mg of S per 100 g.

12

TOTAL SULFUR<sup>5</sup>—TENTATIVE

(For sulfured products and for samples containing little ash or an acidic ash.)

In casserole as large as can be placed in electric muffle furnace available, place 1–3 g of MgO (1 g for fruit juices, 3 g for heavily sugared products and for dried fruits) or an equivalent quantity of Mg nitrate, 1 g of powdered sucrose, and 50 ml of HNO<sub>3</sub>. Add 5–10 g of the prepared sample, 2(a), (b), (c), or (d). Place the same quantities of the reagents in another casserole for blank. Evaporate on steam bath to pasty consistency. Place casserole in cold electric muffle and gradually heat (not above dull redness) until all N<sub>2</sub>O<sub>4</sub> fumes have been driven off. (All organic matter will have been destroyed.) Cool, dissolve in HCl (1+2.5), and filter. Adjust acidity so that soln contains 0.5–1 g of free HCl, heat to boiling, and add dropwise 5 ml of a 10% BaCl<sub>2</sub> soln. Evaporate to 100 ml, allow to stand overnight, filter, wash, ignite, and weigh the BaSO<sub>4</sub>. Correct result for the BaSO<sub>4</sub> obtained in the blank and report as mg of S per 100 g. (The determination should be made in a room free from S fumes.)

13

CHLORINE IN ASH<sup>6</sup>—TENTATIVE.—See XII, 35 and 37.POTASSIUM<sup>7</sup>—TENTATIVE

14

## PREPARATION OF SOLUTION

Dissolve the ash in HCl. If aliquot is desired, filter into volumetric flask, wash filter thoroly, and make up to volume. Pipet aliquot into beaker, adjust to volume

of 50–75 ml, heat to boiling, and add slight excess of  $\text{NH}_4\text{OH}$  and then sufficient saturated  $\text{NH}_4$  oxalate soln to precipitate all Ca and Al. Continue boiling until precipitate begins to settle. Filter into large Pt dish and wash filter thoroly.

## 15

## DETERMINATION

Evaporate the prepared soln nearly to dryness, add 1 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate to dryness, and ignite to whiteness. Maintain full red heat until residue is perfectly white. Dissolve residue in hot  $\text{H}_2\text{O}$ , using at least 20 ml for each dg of  $\text{K}_2\text{O}$  present; add a few drops of  $\text{HCl}$  and an excess of Pt soln, II, 40(b). Evaporate on water bath to thick paste, avoiding exposure to  $\text{NH}_3$ . Treat residue with 90% alcohol. Filter on dry tared Gooch crucible with an asbestos mat that has been washed thoroly with 90% alcohol and dried at  $100^\circ$  for 30 min. Wash precipitate thoroly with 90% alcohol, both by decantation and on crucible mat, and continue washings after filtrate is colorless, using ca 200 ml of wash solns. Wash 5 or 6 times with 10 ml portions of  $\text{NH}_4\text{Cl}$  soln, II, 40(a), to remove impurities from precipitate. Wash again with four or five 10 ml portions of 90% alcohol and dry precipitate 30 min. at  $100^\circ$ . Weigh, wash again with several 100 ml portions of 90% alcohol, dry, and reweigh until a constant weight of  $\text{K}_2\text{PtCl}_6$  is obtained. Calculate to  $\text{K}_2\text{O}$ . Precipitate should be completely soluble in  $\text{H}_2\text{O}$ .

MANGANESE<sup>8</sup>—TENTATIVE

## 16

## PREPARATION OF SOLUTION

Dissolve the ash in  $\text{HCl}$  (1+2), evaporate to dryness, and heat at  $110^\circ$  for 1 hour to dehydrate any  $\text{SiO}_2$ . Dissolve residue in  $\text{HCl}$  (1+4) and filter into volumetric flask. Wash filter thoroly and make up to volume.

## 17

## DETERMINATION

To an aliquot of the prepared soln add sufficient Br water to oxidize any ferrous Fe to the ferric state. Boil off excess Br. Dilute to 150 ml and heat to boiling. Add sufficient 10%  $\text{NaH}_2\text{PO}_4$  to combine with all the Fe and Al. Add plenty of bromocresol green indicator, and while mixture is gently boiling add 10% freshly prepared  $\text{NaOH}$  soln dropwise to first permanent turbidity or an initial color change in event no Fe or Al compounds are present. Continue neutralization by slowly adding 20% Na acetate to give yellow-green color. Fe and Al phosphates are completely precipitated at pH of 4, at which point bromocresol green indicator is yellow-green.<sup>9</sup> Boil gently 1–2 min. if any precipitate of Al or Fe phosphate forms. Allow to settle, filter, wash carefully, and discard precipitate. To filtrate add 10 ml of the Na acetate and adjust pH to 4.2–4.4 (indicated by a yellow-green color with bromocresol green indicator) by adding  $\text{HCl}$  (1+5) dropwise. Add sufficient Br water to color soln distinctly orange, cover with watch-glass, and boil gently ca 3 min. Take great care to avoid bumping. Allow mixture to settle, add a little more Br water, and again boil gently 1–2 min. Again allow to settle, filter, and wash beaker and filter thoroly. The filtrate is reserved for Ca and Mg determinations. Dissolve hydrated oxide precipitate from filter into original beaker with as little saturated  $\text{SO}_2$  soln as possible. Wash filter paper thoroly with hot  $\text{H}_2\text{O}$ . Boil to remove all odor of  $\text{SO}_2$ , add 10 ml of  $\text{H}_2\text{SO}_4$  and 10–20 ml of  $\text{HNO}_3$ , carefully dilute to 50–75 ml, and heat to boiling, slowly introducing small quantities of  $\text{KIO}_4$  (ca 0.05 g) with spatula until maximum color is produced (ca 0.2 g of periodate is sufficient). Cool, and introduce into volumetric flask. The Mn in the final dilution for colorimetric comparison should be no more than 1 mg per 50 ml. Compare color with standards prepared as directed in XII, 13, except to substitute 10 ml of  $\text{HNO}_3$  for the  $\text{Fe}(\text{NO}_3)_2$ . Report

as percentage of  $\text{Mn}_2\text{O}_3$  by multiplying  $\text{KMnO}_4$  by factor 0.4827. Accurate results may be expected.

#### CALCIUM<sup>10</sup>—TENTATIVE

18

##### *Double Precipitation Method*

Evaporate filtrate from Mn determination, 17, to 100–150 ml. Boil off any Br remaining and adjust pH to 4.4–4.6 (green to green-blue with bromocresol green indicator) by adding 20% Na acetate (pH of 4.4–4.6 is most favorable for precipitation of Ca oxalate). Add sufficient saturated Na oxalate soln dropwise to precipitate all the Ca from the boiling soln, and continue to boil until oxalate begins to settle, or digest 15 min. on steam bath. Allow to settle until clear, filter, and wash precipitate thoroly with hot  $\text{H}_2\text{O}$ . Reserve filtrate and washings for Mg determination. Carefully wash precipitate back into original beaker, heat, and dissolve the oxalate by adding as little HCl as possible. Reprecipitate the Ca by adding  $\text{NH}_4\text{OH}$  (1+9) soln dropwise until pH is again 4.4–4.6 (green to green-blue with bromocresol green indicator). Add slight excess of saturated  $\text{NH}_4$  oxalate soln while still hot. Digest on steam bath 1 hour and set aside until supernatant liquid is clear, preferably overnight. Filter, and wash with hot  $\text{H}_2\text{O}$ . Determine the Ca either gravimetrically or volumetrically by the usual methods (for small quantities the gravimetric method is preferred). Report as  $\text{CaO}$ .

If Mg is not to be determined, precipitate the Ca once from the boiling soln (freed from Fe, Al, and Mn) with saturated  $\text{NH}_4$  oxalate soln, and proceed as directed above, beginning "Digest on steam bath 1 hour."

19

##### *Single Precipitation Method*

Evaporate filtrate and washings from Mn determination, 17, to 200–250 ml. Add 8–10 drops of bromocresol green indicator and sufficient 20% Na acetate to change pH to 4.8–5.0 (blue). Cover with watch-glass and heat to boiling. Precipitate the Ca slowly by adding 3% oxalic acid soln, a drop every 3–5 seconds, until pH is changed back to 4.4–4.6 (optimum for Ca oxalate precipitation) as indicated by appearance of distinct green shade. Change of color will indicate excess of oxalic acid—more would develop yellow tints, showing an undesirable displacement of the pH. Boil 1–2 min. and allow mixture to settle until clear. Filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Determine either gravimetrically or volumetrically as directed in 18.

20

#### MAGNESIUM<sup>11</sup>—TENTATIVE

Add 2–3 drops of HCl to filtrate and washings from Ca determination, 18, and evaporate to 75–100 ml. If quantity of phosphates naturally in sample, or added for purpose of precipitating Fe and Al, is insufficient to precipitate all the Mg expected, add more but avoid a large excess. For this purpose neutralize with 10%  $\text{NH}_4\text{OH}$  until permanent precipitate forms and add sufficient  $\text{NaH}_2\text{PO}_4$  soln to precipitate all Mg present. Dissolve precipitate by slowly adding 10% HCl dropwise. Use as little HCl as possible to obtain complete soln. Use considerable care and patience in next step because  $\text{MgHPO}_4$  begins to precipitate at pH of 6.7–6.8, which is the critical point. Heat soln to gentle boiling and add  $\text{NH}_4\text{OH}$  (1+9) at rate of 4 drops a minute while maintaining a gentle boil until a crystalline precipitate commences to form. (The first precipitate must be crystalline, not gelatinous.) If first precipitate is gelatinous, redissolve it with a little HCl and start precipitation again more slowly. (Stirring assists crystallization, but sides of beaker should not be scratched.) After crystals have formed in considerable numbers hasten the pre-

cipitation. (This treatment gives crystalline  $\text{MgHPO}_4$ .) Continue addition of the dilute  $\text{NH}_4\text{OH}$  until soln is slightly ammoniacal. Allow mixture to cool slightly, then add  $\frac{1}{2}$  the volume of  $\text{NH}_4\text{OH}$  slowly and with constant stirring. Let stand until precipitate has been converted into  $\text{MgNH}_4\text{PO}_4$ , preferably overnight. Filter, and wash carefully with the dilute  $\text{NH}_4\text{OH}$ , until all chlorides have been removed. Dry, and ignite slowly until all the C is consumed. Cover, and ignite intensely. Weigh white  $\text{Mg}_2\text{P}_2\text{O}_7$  and report as  $\text{MgO}$ .  $\text{Mg}_2\text{P}_2\text{O}_7 \times 0.3621 = \text{MgO}$ . (Ignition of dark colored residues with a drop of 20%  $\text{NH}_4\text{NO}_3$  will often improve the color. If the nitrate is added, use care to avoid spattering.)

## 21

ALCOHOL PRECIPITATE<sup>5</sup>—TENTATIVE

To 100 ml of prepared soln, 2(b<sub>1</sub>) or 2(c<sub>1</sub>), in beaker, add 4–8 g of sucrose (1 or 2 lumps of cube sugar) if sugar is not already present, and evaporate to volume of 20–25 ml. If water-insoluble matter separates during evaporation add more sugar. Cool to room temp. and add slowly and with constant stirring 200 ml of alcohol. Allow to stand at least 1 hour, filter on 15 cm qualitative paper, and wash precipitate with alcohol. Do not permit alcohol precipitate to dry before transferring it from paper. Wash precipitate back into original beaker with hot  $\text{H}_2\text{O}$ , rinsing filter paper thoroly. Evaporate soln to ca 20 ml and add 5 ml of  $\text{HCl}$  (1+2.5). If water-insoluble matter separates, stir well and, if necessary, warm slightly to dissolve. Again precipitate with 200 ml of alcohol, allow to stand 1 hour, and filter thru paper. Wash precipitate and paper thoroly with alcohol to remove all  $\text{HCl}$ . Rinse precipitate from filter paper into a Pt dish with hot  $\text{H}_2\text{O}$ , evaporate to dryness on steam bath, dry to constant weight in water oven, and weigh; ignite and weigh again. The loss in weight is the alcohol precipitate.

As the precipitate in many samples is colorless and almost invisible, care must be exercised that none is lost in the dissolving and transferring operations. If the quantity of the alcohol precipitate, as indicated by its volume in the first precipitation, is not excessive, the second filtration may be made thru a Gooch crucible containing a thin asbestos mat. If the alcohol precipitate is very pure and small in quantity it may not be visible at first. In this case, add a small amount of an electrolyte, like  $\text{NaCl}$ , which will flocculate the alcohol precipitate and render it visible.

## 22

PECTIC ACID<sup>8</sup> (DI-GALACTURONIC ACID)—TENTATIVE

Transfer 200 ml aliquot of prepared soln, 2(b<sub>1</sub>) or 2(c<sub>1</sub>), to beaker, add 8–12 g of sucrose (2 or 3 lumps of cube sugar) if soln does not already contain sugar, and evaporate to ca 25 ml. If organic acids are to be determined in filtrate from the pectin, cool, add 3 ml of normal  $\text{H}_2\text{SO}_4$ , and immediately add with constant stirring 200 ml of alcohol, allow precipitate formed to settle, filter on 15 cm qualitative paper, and wash with alcohol. If organic acids are not to be determined, omit addition of  $\text{H}_2\text{SO}_4$ . Transfer precipitate to original beaker with hot  $\text{H}_2\text{O}$ , evaporate to ca 40 ml, and cool to 25° or below. If water-insoluble matter separates during evaporation, stir vigorously, and if necessary add a few drops of  $\text{HCl}$  (1+2.5), and warm; then cool again. Dilute 2–5 ml of 10%  $\text{NaOH}$ , depending upon volume of the precipitate, to 50 ml, and add to soln of the alcoholic precipitate. Allow to stand 15 min., add 40 ml of  $\text{H}_2\text{O}$  and 10 ml of  $\text{HCl}$  (1+2.5), and boil 5 min. Filter, and wash precipitate of pectic acid with hot  $\text{H}_2\text{O}$ . (This filtration should be rapid and the filtrate clear. If filtrate is cloudy or of colloidal nature, reject the determination. Colloidal filtrates are due to insufficient alkali or to saponification at too high a temp., or both. In such cases, repeat determination, using more alkali and keeping temp. low.) Wash precipitate of pectic acid back into beaker, adjust to volume of 40 ml, cool to below 25°, and repeat the saponification with the dilute  $\text{NaOH}$  soln,

the precipitation with the dilute HCl, and the boiling as above described. Again filter and wash precipitate of pectic acid with hot H<sub>2</sub>O, but only to point where test of filtrate shows negligible quantity of acid. (Not more than 500 ml of total filtrate should be necessary.) Wash the pectic acid into Pt dish and dry on steam bath and finally in water oven to constant weight. Weigh, ignite, and weigh again. The loss in weight is pectic acid.

## 23

## PROTEIN—OFFICIAL

Proceed as directed under II, 21, 22, or 23, using 5 g of jelly or other fruit product containing a large quantity of sugar, or 10 g of juice or fresh fruit, and a larger quantity of the H<sub>2</sub>SO<sub>4</sub> if necessary for complete digestion. Percentage of N  $\times 6.25$  = percentage of protein.

## 24

## TOTAL ACIDITY—OFFICIAL

Dilute 10 g of prepared juice, 2(a), or 25 ml of prepared soln, 2(b) or 2(c), with recently boiled H<sub>2</sub>O to ca 250 ml, or less if sample is not highly colored. Titrate with 0.1 N alkali, using phenolphthalein indicator. With highly colored products, instead of phenolphthalein soln use azolitmin soln or phenolphthalein powder, XV, 22, on a spot plate. Report as ml of 0.1 N alkali per 100 g or 100 ml of original material.

## 25

## VOLATILE ACIDS—OFFICIAL

Dissolve 10 g of the sample, dilute to 25 ml, and distil in current of steam as directed under XV, 24. 1 ml of 0.1 N alkali = 0.0060 g of acetic acid.

TOTAL TARTARIC ACID<sup>12</sup>*Bi-tartrate Method—Tentative*

## 26

## PREPARATION OF SAMPLE

Choose a quantity of sample whose titratable acidity in terms of normal acid does not exceed 3 ml. Designate as "A" the ml of normal alkali required to neutralize quantity of sample chosen. In no case should solids content exceed 20 g (200 ml of sample soln of a jam or jelly). Adjust volume of sample to ca 35 ml either by evaporation or by the addition of H<sub>2</sub>O, add 3 ml normal H<sub>2</sub>SO<sub>4</sub>, and heat to 50°. Pour adjusted sample into 250 ml volumetric flask, rinse with 10 ml of hot H<sub>2</sub>O and finally with alcohol, cool, dilute to mark with alcohol, shake, and filter thru folded paper (cover funnel with watch-glass). Pipet 200 ml of the filtrate into centrifuge bottle.

If sample contains alcohol, saponification is necessary. Adjust volume to 35 ml, add "A" + 3 ml of normal KOH, heat to ca 60°, and allow to stand overnight. Add "A" + 6 ml of normal H<sub>2</sub>SO<sub>4</sub> and transfer to 250 ml volumetric flask, as described previously. Filter, and pipet 200 ml of filtrate into centrifuge bottle.

## 27

## DETERMINATION

To the soln in centrifuge bottle add quantity of Pb acetate soln equal to "A" + 3 ml, or in case saponification was made, "A" + 6. Shake vigorously for 2 min., and centrifuge at ca 1000 r.p.m. for 15 min. Prepare Pb acetate soln by dissolving 75 g of normal Pb acetate in H<sub>2</sub>O acidulated with 1 ml of glacial acetic acid and diluting to 250 ml with H<sub>2</sub>O. Carefully decant supernatant liquid from the precipitated Pb salts and test with small quantity of the Pb soln. If precipitate is formed, return mixture to centrifuge bottle, add more Pb soln, shake, and again centrifuge. If



sediment lifts, repeat centrifuging, increasing speed and time. Allow to drain thoroly by inverting bottle for several minutes. To material in centrifuge bottle add 200 ml of 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To the Pb salts in centrifuge bottle add ca 150 ml of  $H_2O$ , shake thoroly, and pass in  $H_2S$  to saturation. Transfer to 250 ml volumetric flask, dilute to mark with  $H_2O$ , and filter thru folded paper. Transfer 200 ml of clear filtrate to 400 ml beaker, and evaporate on gauze to 20 ml over small flame. Neutralize with normal potassium hydroxide, using phenolphthalein indicator, and add 5 drops of the alkali in excess. Add 2 ml of glacial acetic acid and 80 ml of 95% alcohol slowly and with constant stirring. Chill in ice bath, stir vigorously 2 min., and place in refrigerator overnight. Decant supernatant liquid onto thin pad of asbestos in Gooch crucible with removable bottom, leaving ca 25 ml in beaker. To contents of beaker add ca 0.3 g of dry purified asbestos. Mix thoroly and wash into crucible with the cold filtrate. Finally wash beaker and crucible with 3 portions of 15 ml each of ice-cold 80% alcohol, sucking crucible dry each time. Transfer pad and precipitate to original beaker with ca 100 ml of hot  $H_2O$ , heat almost to boiling, and titrate with 0.1 N alkali, using phenolphthalein indicator. 1 ml of 0.1 N alkali = 0.015 g of tartaric acid.

#### *Racemate Method*<sup>12</sup> (Kling)—Tentative

28

#### REAGENTS

(a) *Diammonium-citrate soln.*—Dissolve 29 g of citric acid in ca 200 ml of  $H_2O$  and carefully neutralize with dilute  $NH_4OH$  soln, using methyl red indicator. Add 14.5 g of citric acid, dilute to 1 liter, and filter.

(b) *Ammonium-laevo-tartrate soln.*—Dissolve 3.2 g of ammonium-laevo-tartrate free of the dextro-modification in  $H_2O$ , dilute to 200 ml, and filter. Add 1 ml of formalin as a preservative.

(c) *Calcium acetate soln.*—Dissolve 16 g of  $CaCO_3$  in 120 ml of glacial acetic acid diluted with  $H_2O$ , dilute to 1 liter, and filter.

(d) *Dilute hydrochloric acid soln.*—Dilute 34 ml of  $HCl$  with  $H_2O$  to 1 liter.

(e) *Calcium-sodium acetate soln.*—Dissolve 5 g of  $CaCO_3$  in 20 g of acetic acid, add 100 g of Na acetate, dilute to 1 liter, and filter.

(f) *Standard potassium permanganate soln.*—Dissolve 6.9745 g of purest  $KMnO_4$  in  $H_2O$  and dilute to 1 liter. Standardize soln against a soln of pure tartaric acid of known titer in same manner as in final titration. 1 ml of  $KMnO_4$  soln = nearly 0.005 g of tartaric acid.

(g) *Standard oxalic acid soln.*—Dissolve 13.8793 g of purest oxalic acid in  $H_2O$  and dilute to 1 liter. Titrate against the standard  $KMnO_4$  soln.

29

#### DETERMINATION

Using the prepared sample, 26, proceed as directed under 27 thru the decomposition of the Pb salts with  $H_2S$ , dilution to 250 ml, and filtration. Pipet 200 ml of the clear filtrate into 400 ml beaker and evaporate to ca 100 ml. Add 50 ml of  $H_2O$ , 15 ml of the diammonium-citrate soln, 25 ml of the ammonium-laevo-tartrate soln, and 20 ml of the Ca acetate soln. Stir vigorously until Ca racemate begins to precipitate and allow to stand overnight at room temp. Decant onto thin, tightly-tamped pad of asbestos in Gooch crucible with removable bottom and transfer precipitate to Gooch with portion of the filtrate. Wash contents of crucible 5 times with  $H_2O$ , filling crucible about half full and sucking dry each time. Treat precipitate and mat, after removal from Gooch, with 20 ml of the dilute  $HCl$  soln, and wash crucible thoroly with  $H_2O$ . Adjust volume of the soln to ca 150 ml with  $H_2O$ , add 50 ml of the Ca-Na acetate soln, and heat to ca 80°. Cool soln, stir vigorously, and

allow to stand at least 4 hours, stirring occasionally. Filter, and wash as directed in the first operation. Transfer pad and precipitate to casserole with 150 ml of  $\text{H}_2\text{O}$ , add 50 ml of  $\text{H}_2\text{SO}_4$  (1+9), and heat to  $80^\circ$ . Immediately add the standard  $\text{KMnO}_4$  soln until excess is indicated. Again heat to  $80^\circ$ , add an additional 5 ml of the permanganate soln, and allow to stand ca 1 min. After reheating to  $80^\circ$ , immediately add 10 ml of the standard oxalic acid soln and titrate back with the  $\text{KMnO}_4$  soln.  $\text{KMnO}_4$  soln required for oxidation (ml)  $\times 0.005 \div 2$  = tartaric acid in aliquot.

CITRIC ACID<sup>10</sup>—TENTATIVE

30

## REAGENTS

- (a) *Potassium bromide soln.*—Dissolve 15 g of KBr in 40 ml of  $\text{H}_2\text{O}$ .  
 (b) *Potassium permanganate soln.*—Dissolve 5 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.  
 (c) *Ferrous sulfate soln.*—Dissolve 40 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$  containing 1 ml of  $\text{H}_2\text{SO}_4$ .  
 (d) *Lead acetate soln.*—Dissolve 75 g of normal Pb acetate in  $\text{H}_2\text{O}$ , add 1 ml of glacial acetic acid, and dilute to 250 ml.

31

## DETERMINATION

To the prepared soln, 26, in centrifuge bottle, add quantity of the Pb acetate soln equal to "A"+3, or in case saponification was made, "A"+6. Shake vigorously for 2 min. and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from precipitated Pb salts and test with small quantity of the Pb soln; if precipitate is formed, return to centrifuge bottle, add more Pb soln, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow to drain thoroly by inverting bottle for several minutes. To material in centrifuge bottle add 200 ml of 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To the Pb salts in centrifuge bottle add ca 150 ml of  $\text{H}_2\text{O}$ , shake thoroly, and pass in  $\text{H}_2\text{S}$  to saturation. Transfer to a 250 ml volumetric flask, dilute to mark with  $\text{H}_2\text{O}$ , and filter thru folded paper. Pipet 200 ml of filtrate into 500 ml Erlenmeyer flask, and evaporate to ca 75 ml. Cool, and add 10 ml of  $\text{H}_2\text{SO}_4$  (1+1) and 5 ml of the KBr soln. Heat mixture to  $48\text{--}50^\circ$ , allow to stand 5 min., and add 50 ml of the  $\text{KMnO}_4$  soln. Mix, and allow to stand 1 min. Stopper flask, shake ca 1 min., and allow to stand 3 min. (During this time there should be a heavy deposit of  $\text{MnO}_2$ ; if necessary, add more  $\text{KMnO}_4$  to assure excess of the oxidizing agent. If at any time during oxidation the precipitated  $\text{MnO}_2$  disappears, discard determination and repeat, using more  $\text{KMnO}_4$ .) Remove the  $\text{MnO}_2$  with the  $\text{FeSO}_4$  soln (ca 20 ml), cool to ca  $15^\circ$ , stopper flask, shake vigorously several minutes, and place in refrigerator overnight. Decant supernatant liquid onto thin, tightly tamped pad of asbestos in Gooch crucible (it is important that filtration be completed as quickly as possible). Note volume of filtrate (S in formula) and use filtrate to transfer precipitate to crucible. Wash contents of crucible at once with 50 ml of ice-cold  $\text{H}_2\text{O}$ . Dry by aspirating with dry air or in vacuum desiccator and weigh. Remove pentabromacetone by treating contents of crucible with three portions of 20 ml each of alcohol and three portions of 20 ml each of ether. Again dry and weigh. Difference in two weights = weight of pentabromacetone. Calculate citric acid in aliquot by following formula:

$$X = 0.445P + 0.018S, \text{ in which}$$

X = mg of citric acid in aliquot,

P = weight of pentabromacetone (mg), and

S = volume of filtrate (ml).

For drying the pentabromacetone by aspiration, use apparatus shown in Fig. 32.

- A, Gooch crucible (28 mm diam.) loosely packed with cotton;  
 B, Gooch crucible (35 mm diam.) for pentabromacetone; and  
 C, Suction flask (ca 500 ml capacity).

Dry the air by passing it thru  $\text{H}_2\text{SO}_4$  and soda-lime, and finally filter thru cotton. Cool the air entering drying train by passing it thru spiral condenser cooled with  $\text{H}_2\text{O}$ .

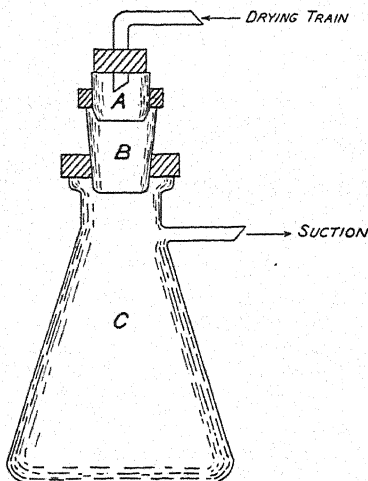


FIG. 32.—APPARATUS FOR DRYING PENTABROMACETONE BY ASPIRATION

Allow crucible, B, containing the pentabromacetone, to remain under suction ca 1 min. to remove surface moisture before placing in apparatus. If air does not pass thru freely, place crucible in desiccator for short time. Maintain slow uniform flow of air by just "cracking" the suction. Dry until loss in weight does not exceed few tenths of a mg, making first weighing after 20 min.

#### LAEVO-MALIC ACID<sup>14</sup>—TENTATIVE

(The method is empirical, therefore all the directions must be rigidly followed, particularly with respect to dilutions. The substitution of volumetric flasks of different capacities than those specified is not permissible.)

#### 32

#### PREPARATION OF SAMPLE

Proceed as directed under 26, omitting addition of the 3 ml of normal  $\text{H}_2\text{SO}_4$  to adjusted sample. In case of saponification add "A" +3 ml normal  $\text{H}_2\text{SO}_4$  to saponified material instead of "A" +6 ml.

#### 33

#### REAGENTS

(a) *Lead acetate soln.*—Dissolve 40 g of normal Pb acetate in  $\text{H}_2\text{O}$ , add 0.5 ml of glacial acetic acid, and dilute to 100 ml.

(b) *Standard tribasic lead acetate soln.*—Prepare soln from tribasic Pb acetate described below. To 5 g of the salt in 500 ml Erlenmeyer flask, add 200 ml of  $\text{H}_2\text{O}$  and shake vigorously. Neutralize 3 ml of normal  $\text{H}_2\text{SO}_4$ , diluted with 200 ml of  $\text{H}_2\text{O}$ , with the soln, using methyl red as indicator. Note volume of Pb soln required. In determination use 2 ml in excess of this quantity. (Solution should be freshly prepared.)

(c) *Tribasic lead acetate.*—Dissolve 82 g of normal Pb acetate in 170 ml of  $\text{H}_2\text{O}$ . Prepare 100 ml of dilute  $\text{NH}_4\text{OH}$  containing 5.8 g of  $\text{NH}_3$  as determined by titration (methyl red). Heat the solns to  $60^\circ$ , mix thoroly, and allow to stand overnight. Shake vigorously to break up precipitate, and filter on Büchner funnel; wash once with  $\text{H}_2\text{O}$  and suck dry, then twice with alcohol, and finally with ether. Allow to dry in air.

#### 34

#### DETERMINATION

To material in centrifuge bottle, add ca 75 mg of tartaric acid and quantity of the Pb acetate soln equal to "A" ("A" +3 ml in case saponification was made),

shake vigorously 2 min. and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from precipitated Pb salts and test with small quantity of the Pb acetate soln. If precipitate is formed, return to centrifuge bottle, add more Pb acetate, shake, and again centrifuge. If the sediment lifts, repeat centrifuging, increasing speed and time. Allow to drain thoroly by inverting bottle for several minutes. Add ca 200 ml of 80% alcohol, shake vigorously, again centrifuge, decant, and drain. To Pb salts add ca 150 ml of  $H_2O$ , shake vigorously, and pass in rapid stream of  $H_2S$  to saturation. Stopper bottle and shake ca 1 min. Transfer to 250 ml volumetric flask with  $H_2O$ , dilute to mark, shake, and filter thru folded paper. Pipet 220 ml of the filtrate into 600 ml beaker and evaporate on gauze to ca 50 ml. Cool, neutralize with normal KOH (phenolphthalein), and add 5 drops in excess. Add 2 ml of glacial acetic acid and transfer with alcohol to 250 ml volumetric flask. Add alcohol to mark, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads and cool to 15°. Stopper flask, shake vigorously 10 min., and place in refrigerator for 30 min. Again shake 10 min. and filter thru a folded paper. Pipet 220 ml of clear filtrate into centrifuge bottle, add Pb acetate soln equal to "A" ("A" + 3 ml in case of saponification), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml of 80% alcohol, shake, centrifuge, decant, and drain. Transfer the Pb salts to 500 ml Erlenmeyer flask with ca 175 ml of  $H_2O$ . Add 3 ml of normal  $H_2SO_4$ , heat to boiling, and add 1 ml of acetic acid soln (5+95) and the quantity of the standard tribasic Pb acetate soln previously determined, 33. Boil mixture 5 min., cool to room temp., transfer to 250 ml volumetric flask with  $H_2O$ , dilute to mark, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads, cool to 15°, shake vigorously 5 min., and place in refrigerator for 30 min. Again shake 5 min. and filter thru folded paper. Saturate clear filtrate with  $H_2S$ , shake vigorously, and filter.

*Polarization.*—Evaporate 225 ml of the clear filtrate over gauze to ca 10 ml, neutralize with normal KOH (phenolphthalein), make slightly acid with the dilute acetic acid, and evaporate to ca 5 ml. Transfer to 25–27.5 ml Giles flask with  $H_2O$ , dilute to 27.5 ml mark, shake, and pour into small Erlenmeyer flask. If Giles flask is not available, use 25 ml measuring cylinder, dilute to mark, and add 2.5 ml of  $H_2O$  from buret. Add small handful of glass beads and 4 g of powdered uranyl acetate, shake vigorously for 10 min., and filter. (As the uranium-malic complex is sensitive to light, while shaking wrap flask in a towel and protect from light as much as possible during filtration and polarization.) Polarize in 200 mm tube at 20°, using white light. After filling tube, release tension on glass disks by slightly loosening caps, and allow to remain at 20° for at least 30 min. before making readings.

Ventzke reading  $\times$  factor 30.1 = mg of laevo-malic acid contained in the portion taken for analysis. If control for adjusting to standard temp. 20° is lacking, determine temp. of polariscope and at this temp. prepare the soln of the uranium complex as described above. Make readings after allowing tube to remain in trough of instrument 30 min.

#### INACTIVE MALIC ACID<sup>15</sup>—TENTATIVE

(The method is empirical, therefore all the directions must be rigidly followed, particularly with respect to dilutions. The substitution of volumetric flasks of different capacities than those specified is not permissible.)

#### 35

#### PREPARATION OF SAMPLE

Subject 2 portions of the sample to the isolation procedure, 37(a); use one portion for determination of laevo-malic acid (polarization) and the other for total malic acid, laevo + inactive (oxidation). Choose a quantity of sample that has a titratable acidity not exceeding 150 mg of acid calculated as malic acid. Designate as "A"

the ml of normal alkali required to neutralize the quantity of sample chosen. In no case should solids content exceed 20 g (200 ml of sample soln of a jam or jelly).

Adjust volume of sample to ca 35 ml either by evaporation or by addition of  $H_2O$ , pour into 250 ml volumetric flask, rinse with 10 ml of hot  $H_2O$  and finally with 95% alcohol, and dilute to mark with the alcohol. Shake, and filter thru folded paper, draining thoroly and covering funnel with watch-glass. Pipet 225 ml of filtrate into centrifuge bottle.

Proceed as directed under 26, beginning "Designate as 'A'."

## 36

## REAGENTS

Use reagents described under 33 and in addition—

(d) *Potassium permanganate soln.*—Dissolve 14.5214 g of the purest  $KMnO_4$  in  $H_2O$  and dilute to 1 liter. Standardize soln as follows: Pipet 50 ml of the oxalic acid soln (e) into 600 ml beaker and add 70 ml of  $H_2O$  and 10 ml of  $H_2SO_4$  (1+1). Heat to  $80^\circ$ , immediately run in the permanganate soln until faint pink color is produced, again heat to  $80^\circ$ , and finish titration. Fifty ml of the permanganate soln should be equivalent to 50 ml of the oxalic acid soln; 1 ml of the oxalic acid soln  $\approx$  5 mg of malic acid (laevo or inactive).

(e) *Oxalic acid soln.*—Dissolve 28.7556 g of the purest  $H_2C_2O_4 \cdot 2H_2O$  in  $H_2O$  and dilute to 1 liter.

## 37

## DETERMINATION

(a) *Isolation of total malic acid.*—To the soln in centrifuge bottle add ca 25 mg of citric acid and a quantity of the Pb acetate soln equal to "A" ("A" + 3 ml in case of saponification), shake vigorously for 2 min., and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from the precipitated Pb salts and test with small quantity of the Pb acetate soln; if a precipitate is formed, return to centrifuge bottle, add more Pb acetate, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow precipitate to drain thoroly by inverting bottle for several minutes. Add 200 ml of 80% alcohol and shake vigorously; again centrifuge, decant, and drain. Add ca 150 ml of  $H_2O$  to Pb salts, shake vigorously, and pass in rapid stream of  $H_2S$  to saturation. Stopper bottle and shake ca 1 min. Transfer mixture to 250 ml volumetric flask with  $H_2O$ , make to mark, shake, and filter. Pipet 225 ml of filtrate into 600 ml beaker, and evaporate to ca 100 ml to expel  $H_2S$ . Transfer to 250 ml volumetric flask with  $H_2O$ . (Volume in flask should be ca 200 ml.) Add 5 ml of acetic acid (1+9), and same quantity of Pb acetate soln previously used. Shake vigorously, dilute to mark with  $H_2O$ , and filter. Into the clear filtrate pass rapid stream of  $H_2S$  to saturation, stopper flask, shake vigorously, and filter. Pipet 225 ml of the filtrate into 600 ml beaker, add ca 75 mg of tartaric acid, and evaporate on gauze to ca 50 ml. Cool, neutralize with normal *potassium* hydroxide (phenolphthalein) and add 5 drops in excess. Add 2 ml of glacial acetic acid and transfer mixture to 250 ml volumetric flask with alcohol. Dilute to mark with alcohol, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads and cool to  $15^\circ$ . Stopper flask, shake vigorously for 10 min. and place in refrigerator for 30 min. Again shake 10 min. and filter thru folded paper. Adjust clear filtrate to  $20^\circ$  and pipet 225 ml into centrifuge bottle. Add Pb acetate soln equal to "A" ("A" + 3 ml in case of saponification), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml of 80% alcohol, shake, centrifuge, decant, and drain. Transfer the Pb salts to 500 ml Erlenmeyer flask with ca 175 ml of  $H_2O$ . Add 3 ml of normal  $H_2SO_4$  and heat to boiling; add 1 ml of acetic acid (5+95) and the quantity of the standard tribasic Pb acetate soln previously

determined under 33. Boil mixture 5 min., cool to room temp., transfer to 250 ml volumetric flask with  $\text{H}_2\text{O}$ , make to mark, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads, cool to ca  $15^\circ$ , shake vigorously 5 min., and place in refrigerator for 30 min. Again shake 5 min. and filter thru folded paper. Saturate clear filtrate with  $\text{H}_2\text{S}$ , shake vigorously, and filter. Use one of the two portions for polarization and the other for oxidation.

(b) *Polarization*.—Evaporate 225 ml of the clear soln over gauze to ca 10 ml and proceed as directed under polarization (laevo-malic acid), 34.

*Ventzke reading*  $\times$  factor 10.2 = mg of laevo-malic acid contained in the aliquot ("1" in the formula).

(c) *Oxidation*.—Evaporate 225 ml of the clear soln to ca 10 ml to expel last traces of alcohol, dilute to ca 120 ml with  $\text{H}_2\text{O}$ , and add 10 ml of a 30%  $\text{NaOH}$  soln and 25 ml of the  $\text{KMnO}_4$  soln. Heat to ca  $80^\circ$  and place in boiling water bath for 30 min. Add 25 ml of the oxalic acid soln and 10 ml of  $\text{H}_2\text{SO}_4$  (1+1), stirring vigorously. Adjust temp. to  $80^\circ$ , and titrate to faint pink color with the permanganate soln. Again heat to  $80^\circ$  and finish titration. Quantity of permanganate used (ml)  $\times 5$  = total oxidizable material (as malic acid) present in aliquot ("t" in formula).

(d) *Calculation*.—Calculate the inactive malic acid "X" (mg) in portion taken for analysis by following formula:

$X = 4(t - 5 - l)$ , in which

$t$  = total oxidizable material (mg) as malic acid;

$l$  = laevo-malic acid (mg);

5 = correction factor for quantity of non-malic material (mg) as malic acid;

4 = factor for reverting inactive malic acid in aliquot back to quantity of inactive acid in sample taken for analysis.

## 38

## PHOSPHORIC ACID

*Gravimetric Method—Official*.—See XV, 19.

*Colorimetric Method*<sup>16</sup>—Tentative

## 39

## REAGENTS

(a) *Molybdenum blue*.—Place 9.78 g of  $\text{MoO}_3$  (99.5–100%) in 500 ml Kjeldahl flask, add ca 150 ml of  $\text{H}_2\text{SO}_4$  (36N  $\pm$  0.5N), and heat with gentle mixing until soln is complete. Cool to  $150^\circ$ . Weigh, on small watch-glass, 0.440 g of very finely powdered molybdenum metal (99.5–100%) and transfer to Kjeldahl flask by sliding watch-glass down neck of flask. Keep at  $140$ – $150^\circ$  and mix vigorously until Mo is dissolved (some larger particles may remain). Cool, transfer to 250 ml volumetric flask, rinse the Kjeldahl with  $\text{H}_2\text{SO}_4$ , and transfer rinsings to the volumetric flask. Finally fill flask to 250 ml with  $\text{H}_2\text{SO}_4$  and mix well. Dilute 10 ml of this reagent with  $\text{H}_2\text{O}$  and titrate with 0.1 N  $\text{KMnO}_4$  to a pink color that persists for a minute. The reagent should be 0.11 N  $\pm$  0.001; if less than 0.109 N add a calculated quantity of Mo and dissolve by reheating in a Kjeldahl flask to  $150^\circ$ . Preserve the deep green soln in glass-stoppered bottles, carefully avoiding contamination of any kind.

(b) *Dilute molybdenum blue*.—Using a pipet previously wet inside with  $\text{H}_2\text{O}$  and washed down afterwards with a few ml of  $\text{H}_2\text{O}$ , pipet 10 ml of (a) into ca 60 ml of  $\text{H}_2\text{O}$  in a 100 ml volumetric flask. Mix, cool, fill to mark with  $\text{H}_2\text{O}$ , and mix. As this reagent deteriorates with age, it should not be used 8 or 10 hours after preparation.

(c) *Sodium hydroxide soln*.—Phosphate and arsenate-free. 3.6 N  $\pm$  0.05 N. Should contain not over 0.0005%  $\text{PO}_4$ . Dissolve the  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , using an arsenic-free Pyrex or porcelain vessel, cool, and titrate with standard acid. Preserve in paraffin-

lined container. Avoid leaving glass equipment in contact with this reagent for any extended period.

(d) *Normal sodium hydroxide*.—From (c) prepare approximately normal NaOH. Preserve in arsenic-free Pyrex or paraffined container fitted with 1 hole stopper bearing a Pyrex medicine dropper.

(e) *Concentrated sulfuric acid*.—Reagent quality.

(f) *Normal sulfuric acid*.—Approximately. Dilute 30 ml of (e) to 1 liter.

(g) *Concentrated nitric acid*.—Reagent quality.

(h) *Perchloric acid*.—60%, reagent quality.

(i) *Sodium alizarin sulfonate soln*.—Dissolve 0.20 g of sodium alizarin monosulfonate in 100 ml of H<sub>2</sub>O and filter. Preserve in indicator bottle.

(j) *Standard phosphate soln*.—0.05 mg P<sub>2</sub>O<sub>5</sub> per ml. Dissolve 0.1917 g of pure dry KH<sub>2</sub>PO<sub>4</sub> in ca 200 ml of H<sub>2</sub>O, and add 10 ml of normal H<sub>2</sub>SO<sub>4</sub> and 6 drops of 0.1 N KMnO<sub>4</sub>. Dilute to exactly 2 liters. This soln keeps indefinitely in well-stoppered Pyrex bottle.<sup>2</sup>

(k) *Glass beads*.—Boil a supply of small glass beads (2 or 3 mm in diameter) in aqua regia, wash clean with H<sub>2</sub>O, and dry.

## 40

## PREPARATION OF SAMPLE

Transfer a portion of sample containing 0.5–2.5 mg of P<sub>2</sub>O<sub>5</sub> to 500 ml Kjeldahl flask. (For determination of P<sub>2</sub>O<sub>5</sub> on water-soluble portion of fruits or fruit juices 25 or 30 ml (equivalent to 3.75 or 4.5 g of fruit) of the sample soln prepared as directed in 2(b) or (c) is a convenient aliquot. For jams and jellies 50 ml of the prepared soln may be taken. If sample has low fruit content, a larger aliquot should be taken.) Add 5 ml of the H<sub>2</sub>SO<sub>4</sub> (e) from pipet or buret, then add 10 ml of the HNO<sub>3</sub> (g) and 5 or 6 small glass beads. Place flask on a digestion rack over a free flame. Protect flask from flame by asbestos mat having a hole of such size that the surface of the H<sub>2</sub>SO<sub>4</sub> will be above the mat. Boil over moderate flame until darkening begins (avoid excessive charring). Add a few ml of the HNO<sub>3</sub> and again boil until slight darkening begins or until SO<sub>3</sub> fumes are evolved from a clear or amber soln. (In the case of jams or jellies, 3 or 4 additions (ca 5 ml each) of HNO<sub>3</sub> may be necessary.) Add to the hot flask 0.5 ml of the HClO<sub>4</sub> and continue fuming for a few minutes. (To avoid violent explosions of HClO<sub>4</sub> in the presence of organic matter do not add over 0.5 ml at one time and then only after practically all organic matter has been removed with HNO<sub>3</sub>, and do not fail to take all precautions advised in the use of HClO<sub>4</sub>.) When digest is water clear or very slightly greenish yellow, cool somewhat and cautiously add 50 ml of H<sub>2</sub>O and boil to fumes to remove traces of HNO<sub>3</sub>. Cool, add ca 25 ml of H<sub>2</sub>O, transfer to 100 ml volumetric flask, mix, cool, make to volume, and mix thoroly.

## 41

## DETERMINATION

Transfer a 20 ml aliquot of sample digest and 0, 2, 4, 6, 8, 10, and 12 ml of the standard phosphate soln to 100 ml volumetric flasks (Kohrausch sugar flasks have been found convenient) marked at 70 ml capacity. To the standards add 30 ml of the normal H<sub>2</sub>SO<sub>4</sub>. To the samples add 20 or 25 ml of H<sub>2</sub>O, and to all flasks add 3 drops of the sodium alizarin sulfonate soln and then exactly 10 ml of the 3.6 N NaOH soln. Adjust the acidity to just yellow by means of the normal H<sub>2</sub>SO<sub>4</sub> and normal NaOH until a single drop of the normal H<sub>2</sub>SO<sub>4</sub> just changes color of the soln to yellow. Dilute to 70 ml and mix by swirling. Place the flasks in a boiling water bath and bring to that temp. With a pipet add exactly 10 ml of the dilute molybdenum blue reagent, directing the stream into the soln (do not allow it to run down

side of flask), mix by swirling, and continue to heat in the boiling water bath for exactly 20 min. Cool rapidly in cold  $H_2O$ , dilute to volume, and mix.

Keep the standards and unknowns at the same temp. by immersing the flasks in a boiling water bath wherein the  $H_2O$  comes above level of soln in flask. A simple water bath may be prepared by placing a  $\frac{1}{4}$ " mesh wire screen in bottom of a 12 or 14" granite pan and filling pan with  $H_2O$  to such a depth that liquid in flasks will be below level of  $H_2O$ . Place pan on a stand and heat with a large Meeker burner with flame so adjusted that it spreads over bottom of pan and keeps entire contents at a gentle rolling boil. Place the flasks only around periphery of the pan and weight with lead rings or otherwise support to prevent tipping. Keep the bath at a rolling boil thruout heating period and add *boiling*  $H_2O$  to the bath from time to time to keep level of  $H_2O$  above level of liquid in flasks. Keep a thermometer in the bath and do not permit a variation of more than  $2^\circ$  between the center and the edge of the pan. Determine color intensity by means of a neutral wedge photometer,<sup>17</sup> using a 1" cell, No. 66 filter, with Jena 0-2 neutral wedge. (Filter 66 is 4.5 mm Corning dark pyrometer red No. 241. With "B & L Smoke C" glass wedge, use filter 65. Filter 65 is the same as 66 plus a half mm of Jena BG18).

The method covers a range up to 0.6 mg of  $P_2O_5$  in the final 100 ml of soln. Make a large scale graph of the standards, plotting mg of  $P_2O_5$  against photometer readings. (Graph paper  $20 \times 36$ " with 10 lines to the inch is convenient.) By means of this plot convert the sample photometer readings to mg of  $P_2O_5$  present in final 100 ml of soln. If preferred, the equation of the line may be calculated as described by Klein and Vorhes<sup>18</sup> and the equation used in conversion.

#### NOTES

The photometer need be calibrated but once for each batch of reagents provided the adjustment is not altered and the temp. of the boiling water bath remains the same. It is advisable, however, to develop one or two standards with each batch of unknowns in order to detect any possible change of conditions.

It will be noted that standardization under these conditions automatically corrects for the blank on reagents, except  $HNO_3$  and  $HClO_4$ . These reagents in the grade specified have not been found to contain significant quantities of arsenic or phosphorus. It is well, however, to determine the digestion blank on these reagents from time to time.

In the analysis of heterogeneous samples, such as lots of fresh fruit, for total  $P_2O_5$ , it may be necessary to digest a larger portion than specified above in order to minimize sampling and weighing error. In that case it is convenient to take double the above sample and double the amount of  $H_2SO_4$  (10 ml). Make the digest to 200 ml, and finally transfer a 20 ml aliquot to 100 ml volumetric flask for color development. The amount of sample digested may be varied to suit the nature of the sample if the final aliquot taken for color development contains not more than 1 ml of  $H_2SO_4$  and not more than 0.6 mg of  $P_2O_5$ .

Iron, nitrate, and arsenic act as interferences in the development of the color. Nitrates are not present in solns prepared as described, and neither iron nor arsenic is ordinarily present in fruit or fruit products in sufficient quantity to constitute an interference. However, if the presence of excessive arsenic or iron is suspected, their interference may be prevented by a procedure used by Zinzadze. Proceed as directed previously to the point, "Adjust acidity to just yellow," after which add 10 ml of exactly normal  $H_2SO_4$  and then 10 ml of 8%  $Na_2SO_4$ , and dilute to 70 ml. Heat in a boiling water bath for an hour. Then again refer to previous directions and continue with "add exactly 10 ml of the dilute molybdenum blue reagent." Standards and blank, of course, must then be treated in exactly the same manner.

42

FREE MINERAL ACIDS—TENTATIVE.—See XXXIII, 82-84.

#### SUCROSE

43

*By Polarization—Official*

Determine by polarizing before and after inversion, as directed under XXXIV, 23, 24, or 28.



44 *By Reducing Sugars Before and After Inversion—Official.—See XXXIV, 29.*

45 REDUCING SUGARS—OFFICIAL.—*See XXXIV, 38* Express results as invert sugar.

46 COMMERCIAL GLUCOSE—OFFICIAL.—*See XXXIV, 31.*

47 DEXTRIN—TENTATIVE

Dissolve 10 g of sample in 100 ml flask and add 20 mg of KF and then about  $\frac{1}{4}$  cake of compressed yeast. Allow fermentation to proceed below 25° for 2–3 hours to prevent excessive foaming and then incubate at 27–30° for 5 days. Clarify soln with basic Pb acetate soln and alumina cream; make up to 100 ml, filter, and polarize in 200 mm tube. A pure fruit jelly will show a dextro or laevo rotation of not more than a few tenths of a degree. If polariscope having Ventzke scale is used and 10% soln is polarized in 200 mm tube, number of degrees read on sugar scale of the instrument  $\times 0.8755$  = percentage of dextrin; or the following formula may be used:

$$\text{Percentage of dextrin} = \frac{C \times 100}{198 \times L \times W}, \text{ in which}$$

$C$  = degrees of circular rotation;

$L$  = length of tube in decimeters; and

$W$  = weight of sample in 1 ml.

#### STARCH

48 *Qualitative Test—Official*

Dilute a portion of sample with H<sub>2</sub>O, heat nearly to boiling, add several ml of H<sub>2</sub>SO<sub>4</sub> (1+9), and then 10% KMnO<sub>4</sub> soln until all color is destroyed. Cool, and test with I soln, XXXIII, 29(f). (Presence of starch is not necessarily indication of its addition as adulterant. It is usually present in small quantity in the apple, and occasionally in other fruits, and unless it is found in the fruit product in considerable quantity its presence may be due to these natural sources.)

#### GELATIN<sup>19</sup>

49 *Qualitative Test—Tentative*

Gelatin in jellies and jams is shown by increased content of N. Precipitate a concentrated soln of jelly or jam with 10 volumes of absolute alcohol and determine N in dried precipitate as directed under II, 21, 22, or 23.

#### AGAR AGAR

50 *Detection by Microscopic Examination<sup>20</sup>—Tentative*

Heat the jelly with H<sub>2</sub>SO<sub>4</sub> (1+18), add a crystal of KMnO<sub>4</sub>, and allow to settle. If agar agar is present, the sediment will be rich in diatoms, which can be detected under microscope. (The diatoms adhere to the glass and are best obtained by pouring out the liquid, washing glass with 2 or 3 drops of alcohol, and transferring alcohol to microscopic slide by means of glass rod.)

51 *Detection by Precipitation—Tentative*

Cover 30 g of the jam or jelly with 270 ml of hot H<sub>2</sub>O, stir until thoroly disintegrated, and boil 3 min. Filter soln while boiling hot thru rapid qualitative filter

paper. In presence of agar agar a precipitate will form upon standing not longer than 24 hours. Filter, wash with cold  $H_2O$ , and dissolve from paper by means of very small quantity of boiling  $H_2O$ . Upon chilling this soln a firm jelly that can be examined by the touch will be formed. This method will detect 0.2% of agar agar with certainty if proportions of jam or jelly and  $H_2O$  specified are strictly followed.

52

ADDED WATER IN GRAPE JUICE<sup>21</sup>—TENTATIVE

(Applicable to white juices only.)

Measure ca 50 ml of *filtered* juice into 2 oz tincture bottle containing a number of short pieces of glass rod. Add ca 1 g of finely powdered purest K acid tartrate, cool to 25°, and shake 1 hour at this temp. (There should be undissolved bitartrate in the juice; if there is not, repeat operation, using more of the salt.) *Immediately* filter juice and titrate 10 ml of filtrate with 0.1 N alkali, using phenolphthalein indicator. In same manner titrate 10 ml of the original filtered juice. An increase in titer of treated sample is index of added  $H_2O$ . Make the two titrations side by side in order to obtain same shade of pink.

For control of temp. during saturation period the following procedure is suggested: Immerse the tightly corked tincture bottle, neck down, into pint Mason jar filled top-full with  $H_2O$  at 25°. Adjust cover, immerse jar in pail of  $H_2O$  at 25° and maintain this temp. for 30 min. Remove jar from  $H_2O$  and immediately wrap in 3 sheets of heavy paper, making each wrapping separately. Place the system in shaker and shake for 1 hour. Ascertain temp ( $t^\circ$  in formula) of the  $H_2O$  in the jar. Determine titers of treated and untreated juices as directed above and calculate volume % of added sugar soln ( $H_2O$ ) by following formula:

$$W = 3.13(b - a) - 15.8 - 2.08(t^\circ - 25), \text{ in which}$$

$W$  = vol. % added  $H_2O$  (20% sugar soln);

$b$  = acidity of treated juice (ml 0.1 N alkali per 100 ml);

$a$  = acidity of original juice (ml 0.1 N alkali per 100 ml); and

$t^\circ$  = temp. of  $H_2O$  in Mason jar after shaking.

[Pure factory juices examined by this method show a small quantity of added  $H_2O$  (1–3%).]

53

METALS.—See XXIX.

54

PRESERVATIVES.—See XXXII.

55

COLORING MATTERS.—See XXI.

56

SWEETENING SUBSTITUTES.—See XXXII, 13, 14, 37, 38.

## SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., 17, 66 (1934).

<sup>2</sup> Ibid., 18, 80 (1935).

<sup>3</sup> Ibid., 6, 34 (1922).

<sup>4</sup> Ibid., 15, 76 (1932).

<sup>5</sup> Ibid., 6, 37 (1922).

<sup>6</sup> Ibid., 11, 216 (1928); 15, 76 (1932).

<sup>7</sup> Ibid., 12, 366 (1929).

<sup>8</sup> Ibid., 14, 466 (1931).

<sup>9</sup> Clark, The Determination of Hydrogen Ions, 3rd ed. (1928).

<sup>10</sup> J. Assoc. Official Agr. Chem., 12, 366 (1929).

<sup>11</sup> Ibid., 14, 473 (1931).

- <sup>12</sup> Bull. soc. chim., 7, 567 (1910); 11, 886 (1912); J. Assoc. Official Agr. Chem., 8, 638 (1925); 13, 103 (1930).  
<sup>13</sup> J. Assoc. Official Agr. Chem., 13, 99 (1930); 14, 64 (1931).  
<sup>14</sup> Ibid., 15, 648 (1932).  
<sup>15</sup> Ibid., 16, 281 (1933).  
<sup>16</sup> Ind. Eng. Chem., Anal. Ed., 7, 116, 227 (1935); J. Assoc. Official Agr. Chem. 22, 131, 167 (1939).  
<sup>17</sup> J. Assoc. Official Agr. Chem., 19, 120 (1936).  
<sup>18</sup> Ibid., 121.  
<sup>19</sup> Chem. Ztg., 19, 552 (1895).  
<sup>20</sup> Z. angew. Mikrosk., 2, 260 (1896); Z. Nahr. Genussm., 21, 185 (1911).  
<sup>21</sup> J. Assoc. Official Agr. Chem., 8, 724 (1925); 9, 38 (1926).

## XXVII. GRAIN AND STOCK FEEDS

1

### PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass thru sieve having circular openings  $1/25''$  (1 mm) in diameter and mix thoroly. If sample cannot be ground, reduce it to as fine a condition as possible.

#### MOISTURE<sup>1</sup>

2

#### *I. Drying with Heat—Official*

Dry to constant weight at  $95-100^{\circ}$  under pressure not to exceed 100 mm of Hg (ca 5 hours), a quantity of the substance representing ca 2 g of dry material. Use covered Al dish at least 50 mm in diameter and not exceeding 40 mm in depth. Report loss in weight as moisture.

#### *II. By Distillation with Toluene—Official*

3

#### APPARATUS

A 250 ml distilling flask of Pyrex or other resistant glass connected by means of "distilling tube receiver" to 20" sealed-in, straight-tube Liebig condenser with delivery tube not over  $5/16''$  in diameter in manner shown, Fig. 33. The receiver, dimensions shown, is made by attaching proper side tube to calibrated section of 5 ml Mohr pipet and sealing outlet. The tube is calibrated in ml by distilling known quantities of  $H_2O$  into the graduated column, and the column of  $H_2O$  may be read to hundredths with reasonable accuracy. Clean tube and condenser with  $K_2Cr_2O_7-H_2SO_4$  mixture, rinse thoroly with  $H_2O$ , then with alcohol, and dry in oven to prevent undue quantity of  $H_2O$  adhering to inner surfaces during determination.

4

#### DETERMINATION

If sample is likely to bump, add enough dry sand to cover bottom of flask. Add sufficient toluene to cover sample completely (ca 75 ml). Weigh and introduce into toluene sufficient sample to give 2-5 ml of  $H_2O$  and connect apparatus as shown, Fig. 33. Fill receiving tube with toluene, pouring it thru top of condenser. Bring to boil and distil slowly, ca 2 drops per second, until most of  $H_2O$  has passed over; then increase rate of distillation to ca 4 drops per second. When all  $H_2O$  is apparently over, wash down condenser by pouring toluene in at top, continuing distillation a short time to ascertain whether any more  $H_2O$  will distil over; if it does, repeat washing down process. If any  $H_2O$  remains in condenser, remove it by brushing down with tube brush attached to Cu wire and saturated with toluene, washing down condenser at same time. (Entire process is usually completed within an hour.) Allow receiving tube to come to room temp. If any drops adhere to sides of tube, force them down by means of Cu wire with end wrapped in rubber band. Read the volume of  $H_2O$  and calculate to percentage.

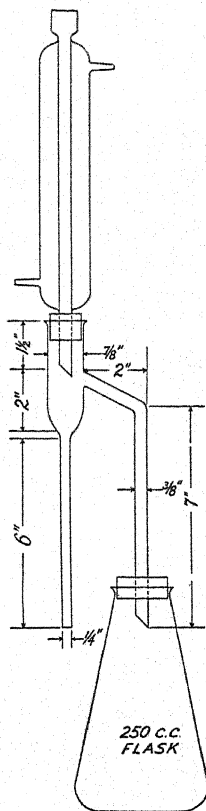


FIG. 33.—APPARATUS USED IN METHOD II FOR THE DETERMINATION OF MOISTURE

*III. Drying without Heat over Sulfuric Acid<sup>2</sup>—Official*

5

## REAGENT

*Sulfuric acid.*—Boil  $\text{H}_2\text{SO}_4$  in large Kjeldahl flask for 4 hours, close mouth of flask with stopper carrying  $\text{CaCl}_2$  tube, and cool.

6

## DETERMINATION

Weigh suitable quantity of sample (2–5 g) into metal dish 5–10 cm in diameter and provided with tightly fitted cover. (If subsequent fat determinations are to be made, fat extraction cones may be used.) Mix substances that dry down to horn-like material with fat-free cotton or other suitable material. Place 200 ml of the fresh  $\text{H}_2\text{SO}_4$  in strong, tight vacuum desiccator. Place dish, uncovered, in desiccator and exhaust by means of vacuum pump to pressure of not more than 10 mm of Hg.

If pump is not available, place 10 ml of ether in small beaker in desiccator and exhaust with water filter pump. Between pump and desiccator interpose empty bottle next to desiccator and bottle of  $\text{H}_2\text{O}$  next to pump. Draw air from desiccator thru the  $\text{H}_2\text{O}$  and turn desiccator stopcock the instant the  $\text{H}_2\text{O}$  begins to rise in tube leading from empty bottle.

Gently rotate desiccator 4 or 5 times during first 12 hours. At end of 24 hours open desiccator, causing incoming air to bubble thru  $\text{H}_2\text{SO}_4$ , place cover on dish, and make first weighing. After weighing place sample in desiccator containing fresh  $\text{H}_2\text{SO}_4$  and exhaust as before. Rotate desiccator several times during the interval and weigh again after suitable period of drying. Repeat this process until weight is constant.

7

*IV. Electric Air-Oven Method<sup>3</sup>—Official*

(Not intended for use when subsequent fat determination is to be made on same sample.)

Regulate electric air oven to  $135^\circ \pm 2^\circ$ . Using low, covered Al dishes, 2, weigh ca 2 g of sample into each dish and shake until contents are evenly distributed. With covers removed, place dishes and covers in oven as quickly as possible and dry samples for 2 hours. After placing covers on dishes transfer them to desiccator to cool. Weigh, and calculate loss in weight as moisture.

8

## ASH—OFFICIAL, FIRST ACTION

Weigh 2 g sample into porcelain crucible and place in muffle furnace previously heated to  $650^\circ$ . Maintain at this temp. 2 hours with an automatic control pyrometer. Transfer crucibles directly to desiccator, cool, and weigh immediately. Report percentage to first decimal place.

9

## CRUDE PROTEIN—OFFICIAL

Determine N as directed under II, 21, 22, or 23, and multiply result by 6.25. In the case of wheat grain multiply the result by 5.7.

QUALITATIVE TESTS FOR PROTEINS<sup>4</sup>—OFFICIAL*Biuret Test*

(Unreliable in presence of glycerol.)

10

## REAGENT

Add slowly with stirring 25 ml of 3% soln of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to 1 liter of 10%  $\text{NaOH}$ . If it is necessary to filter reagent, use glass wool.

11

## DETERMINATION

To 2 or 3 ml of the protein soln add, with shaking, a few drops of the reagent. If characteristic pink or violet color does not develop quickly, allow soln to stand 15 or 20 min. In presence of  $(\text{NH}_4)_2\text{SO}_4$  the addition of NaOH is necessary.

(a) *Osborne's modification*.—This modification of biuret test greatly increases its delicacy. Make the test as described above. Then add 10–20 drops of 95% ethyl alcohol and a piece of solid NaOH (ca 5 g). The alkali “salts out” the small quantity of alcohol, which carries with it the color present, and in this way the presence of small quantities of protein can be detected.

The biuret test is dependent on the peptide grouping,  $-\text{HN} \cdot \text{CO} \cdot \text{NH}-$ , and therefore is given by all proteins. It is also given by certain other compounds containing similar groupings, such as biuret,  $\text{H}_2\text{N} \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{NH}_2$ , and malonamide,  $\text{H}_2\text{N} \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NH}_2$ . Compounds containing one  $-\text{CO} \cdot \text{NH}_2$  and one  $-\text{CSNH}_2$ ,  $-\text{C}(\text{NH})\text{NH}_2$  or  $-\text{CH}_2\text{NH}_2$  similarly joined will also respond to this test.

*Millon's Test*

(Given by all aromatic substances, such as phenol and salicylic acid, which contain a benzene nucleus with a substituted hydroxyl group. In proteins this grouping is furnished by the amino acid tyrosine.)

12

## REAGENT

Dissolve, by gently warming, one part by weight of Hg in two parts by weight of  $\text{HNO}_3$ , sp. gr. 1.42. Dilute soln with two volumes of  $\text{H}_2\text{O}$ . Allow mixture to stand overnight and decant supernatant liquid. The soln contains  $\text{Hg}(\text{NO}_3)_2$  and  $\text{HgNO}_3$ ,  $\text{HNO}_3$ , and some  $\text{HNO}_2$ .

13

## DETERMINATION

Add a few drops of the reagent to 4 or 5 ml of the protein soln in test tube. Warm gently by immersing a few minutes in hot  $\text{H}_2\text{O}$ . A pink or a red color slowly develops and a precipitate usually forms. If substance is a solid, suspend in 3 or 4 ml of  $\text{H}_2\text{O}$  and treat as directed above. Alkaline solns should first be neutralized to avoid precipitation of  $\text{HgO}$ .

*Glyoxylic Acid Test (Hopkins-Cole)*

14

## REAGENT

Add sufficient  $\text{H}_2\text{O}$  to cover liberally 10 g of powdered Mg in large Erlenmeyer flask. Add 250 ml of a cold saturated soln of oxalic acid, keeping flask cool under water tap during addition of acid. After reaction is over, shake mixture and filter. Acidify filtrate with acetic acid and make volume up to 1 liter with  $\text{H}_2\text{O}$ .

15

## DETERMINATION

To 1 or 2 ml of the protein soln in test tube add 3 ml of the reagent and mix thoroly. By means of pipet allow mixture to flow gently down side of second test tube (slightly inclined) containing 5 ml of  $\text{H}_2\text{SO}_4$ . A reddish-violet color forms at junction of fluids, owing to presence of tryptophane in the protein.

16

*Adamkiewicz Test*

Proceed as directed under 15, except to use glacial acetic acid instead of a prepared soln of glyoxylic acid. The color reaction depends on presence of traces of glyoxylic acid formed from the glacial acetic acid.

17

*Xanthoproteic Test*

Add ca 1 ml of  $\text{HNO}_3$  to 3 ml of the protein soln. A white precipitate forms, which on boiling assumes a yellow color and may dissolve to give a yellow soln. Cool, and make slightly alkaline by careful addition of 30%  $\text{NaOH}$ . Color changes to deep orange. Color development depends on formation of nitro derivatives attached to benzene nucleus, and in proteins is referable primarily to the amino acids tyrosine and phenylalanine.

## ALBUMINOID NITROGEN—OFFICIAL

18

## REAGENT

*Cupric hydroxide.*—Dissolve 100 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 5 liters of  $\text{H}_2\text{O}$ ; add 2.5 ml of glycerol, and then add 10%  $\text{NaOH}$  soln until liquid is slightly alkaline; filter; rub precipitate in mortar with  $\text{H}_2\text{O}$  containing 5 ml of glycerol per liter; and wash by decantation or filtration until washings are no longer alkaline. Again rub precipitate in mortar with  $\text{H}_2\text{O}$  containing 10% of glycerol, thus preparing a uniform gelatinous mass that can be measured with pipet. Determine approximately quantity of  $\text{Cu}(\text{OH})_2$  in 5 ml by diluting to 50 ml with  $\text{H}_2\text{O}$ , filtering, washing, igniting, and weighing as  $\text{CuO}$ .

19

## DETERMINATION

Place 0.7 g of the sample in beaker, add 100 ml of  $\text{H}_2\text{O}$ , and heat to boiling; or, in case of substances rich in starch, heat on steam bath for 10 min.; add quantity of the reagent that contains ca 0.5 g of the  $\text{Cu}(\text{OH})_2$ , stir thoroly, filter when cold, wash with cold  $\text{H}_2\text{O}$ , and without removing precipitate from filter determine N as directed under II, 21, 22, or 23, adding sufficient  $\text{K}_2\text{S}$  or  $\text{Na}_2\text{S}$  soln, II, 19(f), to precipitate all the Cu and Hg. The filter paper used must be practically free from N. If the material (such as seeds, seed residue, or oil cake) is rich in alkaline phosphates, add 1–2 ml of 10% soln of  $\text{NH}_3$ -free soda alum to decompose alkaline phosphates, then the  $\text{Cu}(\text{OH})_2$ , and mix well by stirring. If this is not done, Cu phosphate and free alkali may be formed and the protein-copper precipitate partially dissolved in the alkaline liquid.

20

## AMIDO NITROGEN—OFFICIAL

Subtract percentage of albuminoid N from percentage of total N to obtain amido N.

## CRUDE FAT OR ETHER EXTRACT

*Direct Method—Official*

21

## REAGENT

*Anhydrous ether.*—Wash commercial ether with 2 or 3 successive portions of  $\text{H}_2\text{O}$ , add solid  $\text{NaOH}$  or  $\text{KOH}$ , and let stand until most of the  $\text{H}_2\text{O}$  has been abstracted from the ether. Decant into dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until there is no further evolution of H gas. Keep ether, thus dehydrated, over metallic Na in loosely stoppered bottles.

22

## DETERMINATION

Large quantities of soluble carbohydrates may interfere with the complete extraction of the fat. In such cases extract with  $\text{H}_2\text{O}$  before proceeding with the determination. Extract ca 2 g of the sample, dried as directed under 2, 6, or 7, with the anhydrous ether for 16 hours. Dry extract at temp. of boiling  $\text{H}_2\text{O}$  for 30 min., cool

in desiccator, and weigh; continue, at 30 min. intervals, this alternate drying and weighing until weight is constant. For most feeds 1–1.5 hours is required.

23

*Indirect Method—Official*

Determine moisture as directed under 2, 6, or 7; then extract dried substance for 16 hours as directed under 22, and dry again. Report loss in weight as ether extract.

## FAT IN DRIED MILK PRODUCTS

24

*Modified Roese-Gottlieb Method<sup>b</sup>—Official, first action*

Weigh 1 g of well-mixed milk powder and transfer immediately to dry Mojonnier extraction flask or dry Rührig tube. Add 8.5 ml of warm  $H_2O$ , cork, and shake vigorously until dissolved. If necessary warm slightly and then cool to room temp. Add 1.5 ml of  $NH_4OH$  and shake thoroly; add 10 ml of alcohol and shake thoroly; add 25 ml of ethyl ether, cork, and shake thoroly; and finally add 25 ml of petroleum benzin and shake as before. Allow ether layer to separate by leaving flask or tube at rest for 20 min. or until upper liquid is practically clear. Draw off as much as possible of the ether fat soln in flask or Al dish. Evaporate on hot plate or steam bath at temp. that effects complete evaporation, but not so high that spattering or vigorous boiling will result. To residue in flask or tube add 4 ml of alcohol and mix thoroly without inserting stopper. Add 15 ml of ethyl ether and shake thoroly; add 15 ml of petroleum benzin and again shake thoroly. Let stand and separate ether layer as before, drawing it off into same flask or dish and evaporating ether.

Make third extraction in exactly same manner as the second, omitting addition of alcohol. If necessary, carefully pour a few ml of  $H_2O$  down side of tube to raise level of aqueous layer, so the ethers may be completely poured off. (At no time should any of aqueous layer be allowed to run into dish.) After ether is entirely evaporated, place dish in Mojonnier oven for 5 min. with temp. at exactly  $135^\circ$ , or in boiling water oven for 30 min., or longer if required to bring it to constant weight.

Remove fat completely with petroleum benzin and dry residue; weigh, and deduct from total weight. Loss in weight = percentage of fat. Finally, correct this weight by blank determination on reagents used.

NOTES.—The time required for each shaking after addition of first portion of alcohol and subsequent additions of either ethyl ether or petroleum benzin should be not less than 30 seconds, and the shaking should be very vigorous. Each time after drawing off the benzin layer the lip of the extraction flask or the spigot should be rinsed with petroleum benzin, and the rinsings allowed to run into the Al dish. The cork should be washed down at least once with petroleum benzin. Petroleum benzin should have a boiling point below  $60^\circ$ , and both petroleum benzin and ethyl ether should be free from residue on evaporation. The official method, XXII, 20, requires the use of a small quick-acting filter for drawing off the ether layer and washing the filter with petroleum benzin. However, if care is used in making the separation, the use of the filter is not absolutely necessary.

CRUDE FIBER<sup>c</sup>—OFFICIAL

25

## REAGENTS

(a) *Sulfuric acid soln.*—Contains 1.25 g of  $H_2SO_4$  per 100 ml.

(b) *Sodium hydroxide soln.*—Contains 1.25 g of  $NaOH$  per 100 ml, free, or nearly so, from  $Na_2CO_3$ .

The strength of these solns must be accurately checked by titration.

(c) *Asbestos.*—Digest on steam bath or at equivalent temp. for at least 8 hours



with an approximately 5% NaOH soln and thoroly wash with hot H<sub>2</sub>O; then digest in a similar manner for 8 hours with HCl (1+3) and again wash thoroly with hot H<sub>2</sub>O. Dry, and ignite at bright red heat.

26

## APPARATUS

(a) *Condenser*.—Use condenser that will maintain constant volume of soln thru-out process of digestion.

(b) *Digestion flasks*.—Use digestion flasks of such size and shape that soln will be not less than 1" nor more than 1.5" in depth. A 700–750 ml Erlenmeyer flask is recommended.

(c) *Filtering cloth*.—Use filtering cloth of such character that no appreciable solid matter passes thru when filtering is rapid. Butcher's linen or dress linen with ca 45 threads to the inch or No. 40 filtering cloth made by National Filter Cloth and Weaving Company, 1717 Dixwell Ave., Hamden, Conn., or its equivalent, may be used.

27

## DETERMINATION

Extract 2 g of the dry material with ordinary ether, or use residue from ether extract determination (22 or 23), and transfer residue, together with ca 0.5 g of asbestos, to digestion flask. (If residue from the ether extract is used and proper quantity of asbestos has already been added, further addition is unnecessary.) Add 200 ml of the boiling H<sub>2</sub>SO<sub>4</sub> soln, immediately connect digestion flask with condenser, and heat. (It is essential that contents of flask come to boiling within 1 min. and that the boiling continue briskly for exactly 30 min.) Rotate flask ca every 5 min. in order to mix charge thoroly. Take care to keep material from remaining on sides of flask out of contact with soln. (A blast of air conducted into flask will serve to reduce frothing of liquid.) At expiration of 30 min. remove flask, immediately filter thru linen in fluted funnel, and wash with boiling H<sub>2</sub>O until washings are no longer acid. Bring a quantity of the NaOH soln to boiling and keep at this temp. under reflux condenser until used. Wash charge and asbestos back into flask with 200 ml of the boiling NaOH soln, using wash bottle marked to deliver 200 ml. (The boiling NaOH soln is conveniently transferred to the wash bottle by means of bent tube thru which liquid is forced by blowing into tube connected with top of the reflux condenser attached to the NaOH flask.) Connect flask with reflux condenser and boil for exactly 30 min., so timing the boiling with the alkali that contents of different flasks will reach boiling point ca 3 min. apart, which permits sufficient time for filtration. At expiration of 30 min. remove flask and immediately filter thru Gooch prepared with asbestos mat, thru alundum crucible, or thru the filtering cloth in fluted funnel. If the filtering cloth is used, thoroly wash residue with boiling H<sub>2</sub>O and transfer it to Gooch crucible prepared with thin but close layer of ignited asbestos. After thoro washing with boiling H<sub>2</sub>O, wash with ca 15 ml of alcohol. Dry crucible and contents at 110° to constant weight. Cool in an efficient desiccator and weigh. Incinerate contents of crucible in electric muffle or over Meker burner at dull red heat until carbonaceous matter has been consumed (ca 20 min.). Cool in desiccator and weigh. Report loss in weight as crude fiber.

28

## REDUCING SUGARS—OFFICIAL

Place 10 g of the material in 250 ml volumetric flask. If substance has acid reaction, add 1–3 g of CaCO<sub>3</sub> to neutralize acidity. Add 125 ml of 50% alcohol by volume, mix thoroly, and boil on steam bath for 1 hour, using small funnel in neck of flask to condense vapor. Cool, and allow mixture to stand several hours, preferably overnight. Make up to volume with neutral 95% alcohol, mix thoroly, and

allow to settle. Pipet 200 ml of supernatant soln into beaker and evaporate on steam bath to volume of 20–30 ml. Do not evaporate to dryness. A little alcohol in residue does no harm. Transfer to 100 ml volumetric flask and rinse beaker thoroly with  $H_2O$ , adding rinsings to contents of flask. Add enough saturated neutral Pb acetate soln (ca 2 ml) to produce flocculent precipitate, shake thoroly, and allow to stand 15 min. Dilute to mark with  $H_2O$ , mix thoroly, and filter thru dry filter. Add sufficient anhydrous  $Na_2CO_3$  or K oxalate to filtrate to precipitate all the Pb, again filter thru dry paper, and test filtrate with a little anhydrous  $Na_2CO_3$  or K oxalate to make sure that all the Pb has been removed.

Proceed as directed under XXXIV, 38, or 50, using a 25 ml aliquot (representing 2 g of sample). Express results as dextrose or invert sugar.

29

## SUCROSE—OFFICIAL

Introduce 50 ml of the prepared soln, 28, into 100 ml volumetric flask, add piece of litmus paper, neutralize with HCl, add 5 ml of HCl, and allow inversion to proceed at room temp. as directed under XXXIV, 24(c). When inversion is complete, transfer soln to beaker, neutralize with  $Na_2CO_3$ , return soln to the 100 ml flask, dilute to mark with  $H_2O$ , filter if necessary, and determine reducing sugars in 50 ml of the soln (representing 2 g of the sample) as directed under 28. Calculate results as invert sugar. Subtract percentage of reducing sugars before inversion from percentage of total sugar after inversion, both calculated as invert sugar, and multiply difference by 0.95 to obtain percentage of sucrose present.

Because the insoluble material of grain or cattle food occupies some space in the flask as originally made up, it is necessary to correct for this volume. To obtain the true quantity of sugars present multiply all results by factor 0.97, as results of a large number of determinations on various materials have shown the average volume of 10 g of material to be 7.5 ml.

## STARCH

30

## I. Direct Acid Hydrolysis—Official

(Intended only for such materials as raw starch, potatoes, etc., including as starch the pentosans and other carbohydrate bodies that undergo hydrolysis and are converted into reducing sugars on boiling with HCl.)

Stir weighed quantity of sample, representing 2.5–3 g of the dry material, in beaker with 50 ml of cold  $H_2O$  for an hour. Transfer to filter and wash with 250 ml of cold  $H_2O$ . Heat insoluble residue 2.5 hours with 200 ml of  $H_2O$  and 20 ml of HCl (sp. gr. 1.125) in flask provided with reflux condenser. Cool, and nearly neutralize with NaOH. Complete volume to 250 ml, filter, and determine dextrose in aliquot of filtrate as directed under XXXIV, 48 or 50. Weight of dextrose obtained  $\times 0.90$  = weight of starch.<sup>8</sup>

## II. Diastase Method with Subsequent Acid Hydrolysis—Official

31

## REAGENT

*Malt extract.*—Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration will be greatly retarded. Prepare an infusion of the freshly ground malt just before it is to be used. For every 80 ml of the malt extract required digest 5 g of the ground malt with 100 ml of  $H_2O$ , at room temp., for 2 hours, or for 20 min. if mixture can be stirred by an electric mixer. Filter to obtain a clear extract (it may be necessary to return first portions of filtrate to filter). Mix the infusion well.

Extract a quantity of the substance (ground to an impalpable powder and representing 4–5 g of the dry material) on close-textured filter with 5 successive portions of 10 ml of ether; wash with 150 ml of alcohol, 10% by volume, and then with a few ml of 95% alcohol. Place residue in beaker with 50 ml of  $H_2O$ , immerse beaker in boiling  $H_2O$ , and stir constantly for 15 min., or until all starch is gelatinized; cool to 55°, add 20 ml of the malt extract, and maintain at this temp. for an hour. Heat again to boiling for a few minutes, cool to 55°, add 20 ml of the malt extract, and maintain at this temp. for an hour, or until residue treated with I soln shows no blue color upon microscopical examination. Cool, make up directly to 250 ml, and filter. Place 200 ml of filtrate in flask, add 20 ml of HCl (sp. gr. 1.125), connect with reflux condenser, and heat in boiling water bath for 2.5 hours. Cool, nearly neutralize with 10% NaOH soln, finish neutralization with  $Na_2CO_3$  soln, and dilute to 500 ml. Mix soln thoroly, pour thru dry filter, and determine dextrose in aliquot as directed under XXXIV, 48 or 50. Conduct blank determination on same volume of the malt extract as used with sample and correct weight of dextrose accordingly. Weight of dextrose obtained  $\times 0.90$  = weight of starch.

. III. In Presence of Interfering Polysaccharides<sup>3</sup>—Official

Weigh 2–6 g (charges of 4 g for linseed meal, or 3 g for dried apple pomace, have been found to be satisfactory) of the well-mixed sample, prepared to pass freely thru sieve not less than 40 mesh to the inch, using smaller charges in the case of materials containing much gel-forming substance. (Weight of starch in charge must not exceed 1.5 g.) Transfer to dry 12.5–15 cm close-textured rapid filtering paper in glass funnel and extract with 5 successive portions of ether, taking for each portion more than enough to cover charge and using cover-glass to retard evaporation. After completing the ether extraction, allow ether to evaporate and then extract charge with 300 ml of dilute alcohol. Concentration of the alcohol may be varied somewhat to suit material under examination. For linseed meal use 35% alcohol (by volume) and for dried apple pomace use 25% alcohol. Follow this with several filterfuls of 95% alcohol and finish leaching operations with a second ether extraction. Conduct also a control determination, preferably in duplicate, using a filter paper extracted with alcohol and the same quantity of  $H_2O$  and malt extract as in the determination. (It is convenient to let charge stand overnight at this point to allow ether and alcohol to evaporate, as alcohol must be eliminated before starting digestion with malt; or charge may be dried at ca 75° until alcohol has been eliminated.)

Transfer as much of dry material as possible from filter paper into glass mortar and pulverize all lumps. Transfer both filter paper and sample to 500 ml volumetric flask, add 20–30 ml of  $H_2O$ , and thoroly wet material by vigorous shaking.

Should more cold  $H_2O$  be needed to make material more fluid, calculate quantity of hot  $H_2O$  to be added accordingly, so that total volume allowing for 40 ml of malt soln will not exceed 200 ml. Let stand a few minutes, add 100 ml of actively boiling  $H_2O$ , and thoroly gelatinize in boiling water bath.

Cool to 50° or lower, add 20 ml of malt extract, 31, to controls as well as to charges, and place flasks in temp.-controlled water bath. Keeping mash thoroly mixed, gradually raise temp. to 70° in 20–30 min. Maintain at 70° for 30 min., stirring mixture from time to time, then increase temp. to 80°, and keep it at that temp. for 10 min. Finally heat to boiling point. Keep mixtures well stirred. Cool contents of flasks and water bath to 55°. Add 20 ml of the malt extract, mix well, and hold at 55° for 1 hour, stirring about once every 10 min. At termination of the digestion rapidly increase temp. to above 80°.

Measure out 316 ml of 95% alcohol. Add a portion, a little at a time, to contents of flask, with thoro shaking between additions. After cooling to room temp. adjust volume with  $H_2O$  so that quantity of liquid is 500 ml, making allowance for volume occupied by charge by adding 3 ml of  $H_2O$  for every 4 g of charge present after bringing contents to 500 ml mark. (Determination may be interrupted at this stage for several days, but volume should be readjusted if evaporation has occurred in meantime.) Mix thoroly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter thru dry paper. Test the solid residue for starch, either microscopically or by the I color test, after elimination of alcohol and gelatinization with  $H_2O$ . (If more than the merest trace of starch is found, reject entire determination.) Evaporate exactly 200 ml of filtrate on steam bath to volume of 15–20 ml, or until practically all alcohol has been expelled. Do not allow evaporation to proceed to dryness.

Transfer aqueous residue of starch conversion products to 200 ml volumetric flask with hot  $H_2O$ , using policeman to recover any dextrin that may be present. Allow to cool somewhat, and complete volume to 200 ml. Transfer contents to suitable digestion flask, add 20 ml of  $HCl$  (sp. gr. 1.125), made by diluting 68 ml of strong acid (sp. gr. 1.19, or 37%  $HCl$ ) to 100 ml, and connect flask with reflux condenser. Heat in boiling water bath for 2.5 hours. Cool, and for samples of linseed meal or other material yielding solns which at this stage need further purification, add not more than 1 ml of 10% soln of phosphotungstic acid in 1%  $HCl$ . Mix, and allow to stand at least 15 min. Increase volume with  $H_2O$  to 250 ml in volumetric flask, mix well, and filter thru dry paper. Partially neutralize 200 ml of filtrate while stirring by adding 10 ml of strong soln of caustic soda (44 g of  $NaOH$  per 100 ml of  $H_2O$ ) and nearly complete neutralization with a little powdered anhydrous  $Na_2CO_3$ . Transfer to 250 ml flask with  $H_2O$ , cool to room temp., make up to mark, and thoroly mix. Filter, if necessary, and determine dextrose in 50 ml aliquot of the filtrate, gravimetrically, as directed under XXXIV, 48 or 50. Correct weight of dextrose obtained by subtracting weight of dextrose found for same aliquot of the malt control, and multiply corrected weight of dextrose by 0.90 to obtain weight of starch.

$$\text{Aliquots: Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250}, \text{ or Charge} \times 0.064.$$

#### 34 IV. In Condensed or Dried Milk Products—Qualitative Test<sup>10</sup>—Tentative

Mix ca 2 g of sample with 100 ml of  $H_2O$  and boil mixture 2 min. Place a few ml of cooled mixture on spot plate or in test tube and add a drop of I-KI test soln (0.05 g of I and 0.2 g of KI dissolved in 15 ml of  $H_2O$ ). If starch is present, a blue color will be produced.

#### PENTOSANS<sup>11</sup>—OFFICIAL

35

#### REAGENTS

(a) *Hydrochloric acid*.—Contains 12% by weight  $HCl$ . To 1 volume of  $HCl$  add 2 volumes of  $H_2O$ . Determine percentage of acid by titration against standard alkali and adjust to proper strength by dilution or addition of more strong acid, as may be necessary.

(b) *Phloroglucin*.—Dissolve small quantity of phloroglucin in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of  $H_2SO_4$ . A violet color indicates presence of diresorcin. A phloroglucin that gives more than a faint coloration may be purified by following method: Heat in beaker ca 300 ml of the dilute  $HCl$  and 11 g of commercial phloroglucin, added in small quantities at a time,

stirring constantly until it is nearly dissolved. Pour hot soln into sufficient quantity of same HCl (cold) to make volume 1500 ml. Allow to stand at least overnight, preferably several days, to permit diorescin to crystallize. Filter immediately before using. A yellow tint does not interfere with its usefulness. In using, add volume containing required quantity of phloroglucin to distillate.

## 36

## DETERMINATION

Place in a 300 ml distillation flask such a quantity of the sample, 2-5 g, that weight of phloroglucide obtained shall not exceed 0.300 g, together with 100 ml of the dilute HCl and several pieces of recently ignited pumice stone. Place flask on wire gauze, connect with condenser, and heat, rather gently at first, and then regulating so as to distil over 30 ml in ca 10 min. Pass distillate thru small filter paper. Replace the 30 ml distilled by like quantity of the dilute acid, added by means of separatory funnel in such manner as to wash down particles adhering to sides of flask, and continue process until distillate amounts to 360 ml. To total distillate add gradually a quantity of phloroglucin dissolved in the dilute HCl and thoroly stir resulting mixture. (Quantity of phloroglucin used should be about double that of furfural expected. The soln turns yellow, then green, and soon there appears an amorphous greenish precipitate that grows darker rapidly, till it becomes almost black.) Make soln to 400 ml with the dilute HCl and allow to stand overnight.

Collect the amorphous black precipitate in weighed Gooch crucible having an asbestos mat, wash carefully with 150 ml of H<sub>2</sub>O so that the H<sub>2</sub>O is not entirely removed from crucible until the very last, and dry 4 hours at temp. of boiling H<sub>2</sub>O. Cool, and weigh in weighing bottle. The increase in weight is considered to be furfural phloroglucide. To calculate the furfural, pentoses, or pentosans from the phloroglucide, use following formulas given by Kröber:

(1) For a weight of phloroglucide, designated by "*a*" in the following formulas, under 0.03 g:

$$\text{Furfural} = (a + 0.0052) \times 0.5170.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0170.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8949.$$

In preceding and also in following formulas, the factor 0.0052 represents weight of the phloroglucide that remains dissolved in the 400 ml of acid soln.

(2) For a weight of phloroglucide "*a*" between 0.03 and 0.300 g, use Kröber's table, XLIII, 18, or the following formulas:<sup>12</sup>

$$\text{Furfural} = (a + 0.0052) \times 0.5185.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0075.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8866.$$

(3) For a weight of phloroglucide "*a*" over 0.300 g, use following formulas:

$$\text{Furfural} = (a + 0.0052) \times 0.5180.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0026.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8824.$$

## 37

## GALACTAN—TENTATIVE

Extract a convenient quantity of sample, representing 2.5-3 g of the dry material, on hardened filter with 5 successive portions of 10 ml of ether; place extracted residue in beaker, ca 5.5 cm in diameter and 7 cm deep; add 60 ml of HNO<sub>3</sub> (sp. gr. 1.15); and evaporate on steam bath to volume of 20 ml. Let stand 24 hours, add 10 ml of H<sub>2</sub>O, and allow to stand another 24 hours. Pass mixture thru filter, wash

impure mucic acid crystals with 30 ml of  $H_2O$  to remove as much of the  $HNO_3$  as possible, and return filter and contents to original beaker. Add 30 ml of  $(NH_4)_2CO_3$  soln (consisting of 1 part  $(NH_4)_2CO_3$ , 19 parts  $H_2O$ , and 1 part of  $NH_4OH$ ) and heat mixture in water bath, at  $80^\circ$ , for 15 min., with constant stirring. The  $(NH_4)_2CO_3$  combines with the mucic acid, forming soluble  $NH_4$  mucate. Wash filter paper and contents several times with hot  $H_2O$  by decantation, passing washings thru filter paper, to which finally transfer residue, and wash thoroly. Evaporate filtrate to dryness on water bath, avoiding unnecessary heating, which causes decomposition; add 5 ml of  $HNO_3$  (sp. gr. 1.15); stir mixture thoroly; and allow to stand 30 min. Collect precipitated mucic acid on weighed Gooch crucible or other filter; wash with 10-15 ml of  $H_2O$ , then with 60 ml of 95% alcohol, and then a number of times with ether; dry at temp. of boiling  $H_2O$  for 3 hours; and weigh. Multiply weight of mucic acid by 1.33 to convert to galactose, and by 1.20 to convert to galactan.

38

WATER-SOLUBLE ACIDITY<sup>13</sup>—TENTATIVE

Weigh 10 g of sample into shaking bottle, add 200 ml of  $H_2O$ , and shake for 15 min. Filter extract thru folded filter and take 20 ml aliquot (equivalent to 1 g of sample). Dilute with 50 ml of  $H_2O$  and titrate with 0.1 N NaOH, using phenolphthalein indicator. Report results in terms of ml of 0.1 N NaOH required for neutralizing extract from 1 g of material.

FAT ACIDITY IN GRAIN<sup>14</sup>—TENTATIVE

39

## REAGENTS

(a) *Benzene-alcohol-phenolphthalein soln.*—To 1 liter of  $C_6H_6$  add 1 liter of alcohol and 0.4 g of phenolphthalein to form 0.02% of mixture.

(b) *Alcohol-phenolphthalein.*—To 1 liter of alcohol add 0.4 g of phenolphthalein (0.04% soln).

(c) *Potassium hydroxide soln.*—0.0178 N. Carbonate free. 1 ml of this soln contains 1 mg of KOH.

40

## APPARATUS

(a) *Grain mill.*—A suitable mill for grinding small samples.

(b) *Sieve.*—40-mesh.

(c) *Drying oven.*—Capable of maintaining the temp. of boiling  $H_2O$ .

(d) *Fat extraction apparatus.*—A Soxhlet or other suitable type. (Double paper thimbles or alundum R. A. 360 thimbles are suitable for extraction purposes.)

## DETERMINATION

41

I. *Applicable to All Cereal Grains and Flour*

Obtain a representative sample of ca 50 g of the grain (corn 100 g) by hand quartering or by use of a mechanical sampling device. Preferably so grind sample that at least 90% will pass thru a 40-mesh sieve (somewhat coarser grind will not materially affect the results). If sample is too moist to grind readily, dry at a temp. of ca  $100^\circ$  only long enough to remove excess moisture.

Extract a 10 g ( $\pm 0.01$  g) ground sample with petroleum benzin ca 16 hours in the extractor. Start extraction as soon as possible after sample has been ground, and under no circumstances allow ground sample to remain overnight. Completely evaporate solvent from extract on steam bath. Dissolve residue in extraction flask with 50 ml of the benzene-alcohol-phenolphthalein mixture. Titrate dissolved ex-

tract with the KOH to a distinct pink color, or in case of a yellow soln to an orange-pink. If an emulsion forms during titration, dispel it by the addition of a second 50 ml portion of the benzene-alcohol-phenolphthalein soln. The end point should match the color of a soln made by adding 2.5 ml of the 0.01%  $\text{KMnO}_4$  to 50 ml of a  $\text{K}_2\text{Cr}_2\text{O}_7$  soln of the proper strength to match the color of the original soln being titrated. (Add a 0.5%  $\text{K}_2\text{Cr}_2\text{O}_7$  soln dropwise to 50 ml of  $\text{H}_2\text{O}$  until the color is matched. Then add 2.5 ml of the 0.01%  $\text{KMnO}_4$  soln. This final soln may be used as the standard for the titration end point of the extract.) Make blank titration on 50 ml of the benzene-alcohol-phenolphthalein mixture and subtract this value from the titration value of sample. If the additional 50 ml portion of the benzene-alcohol-phenolphthalein soln has been added, double the blank titration. Report fat acidity as number of mg of the KOH required to neutralize the free fatty acids from 100 g of grain (dry basis). Fat acidity =  $10 \times (\text{titration minus blank})$ .

42

### II. Rapid Method for Corn

(Results may be obtained in less than 1 hour.)

Prepare sample as directed in procedure I. Weigh 20 g ( $\pm 0.01$  g) into a 100 ml glass-stoppered flask or bottle. Add exactly 50 ml of  $\text{C}_6\text{H}_6$ , insert stopper, shake a few seconds to saturate the air in flask with benzene vapor, momentarily loosen stopper to release pressure, and replace stopper. Shake flask 30 min. in a mechanical shaker, or periodically by hand for 45 min. Tilt flask and allow meal to settle at that angle for at least 3 min. Carefully decant as much of the liquid as possible into a 15 cm folded filter paper inserted in an 8 cm glass funnel, and cover funnel with cover-glass to minimize evaporation. Collect exactly 25 ml of the filtrate in a 25 ml volumetric flask. Then transfer this filtrate to a 250 ml Florence flask. Refill the volumetric flask to the 25 ml mark with the alcohol containing 0.04% phenolphthalein and transfer to flask containing the benzene extract.

Using a color standard prepared as in procedure I, titrate the extract with the standard KOH soln to a distinct pink color in the case of white corn, and to an orange-pink for yellow corn. If an emulsion forms during titration, dispel it by the addition of 25 ml each of benzene and of the alcohol-phenolphthalein reagent. Run a blank titration on a mixture of 25 ml of  $\text{C}_6\text{H}_6$  and 25 ml of the alcohol-phenolphthalein mixture. If the additional benzene and alcohol have been added, double the blank titration. Report fat acidity as the number of mg of KOH required to neutralize the free fatty acids from 100 g of corn (dry basis). Fat acidity =  $10 \times (\text{titration minus blank})$ , calculated on dry basis.

43

### SALT<sup>10</sup> (QUALITATIVE)—OFFICIAL

Transfer 2 ml of 5% soln of  $\text{AgNO}_3$  to small test tube of 1 cm internal diameter. Carefully add to this liquid an equal volume of the feed, which previously has been ground to pass mm sieve, so that most of sample floats or remains above liquid. Gradually incline tube so that liquid is absorbed. White patches of  $\text{AgCl}$  appear wherever the minutest crystal of salt comes in contact with the soln. These patches may easily be observed with a lens or even with the naked eye.

44

### RICE HULLS IN RICE BRAN<sup>10</sup>—TENTATIVE

Thoroughly mix sample to be examined. Withdraw small portion and grind until it passes thru 60-mesh sieve. Weigh 4 mg on a slide ruled in parallel lines  $1/20''$  apart or transfer to ruled slide after weighing. Add just sufficient chloral hydrate soln (1+1) to fill in under cover-glass, which, preferably, should be square (ca 22 mm).

After cover-glass is in place, warm gently, but do not boil, to eliminate starch masses and clear tissues. Count particles of hull tissue, using microscope having magnification of ca 90 diameters. The high refraction and yellowish green color of hull particles will aid in distinguishing the small pieces not easily recognized by their structure. (In order to avoid duplicate counting, it is well to disregard the particles that extend over the upper line of the strip.) Compare results with those obtained on standards containing known quantities of hulls.

45

OAT HULLS IN OATS AND OAT FEEDS<sup>17</sup>—TENTATIVE

(Results are only approximate.)

Place in 1000 ml beaker 800 ml of H<sub>2</sub>O and 2 g of the sample, previously ground to pass thru sieve having circular openings 1 mm in diameter. Stir vigorously to obtain a centrifugal effect, allow to stand 5 min., and decant supernatant liquid carefully, retaining so far as possible all hull particles. Repeat procedure several times until supernatant liquid becomes clear, or nearly so, and then transfer residue with aid of 150 ml of H<sub>2</sub>O to 300 ml beaker. Add 5 drops of HCl and boil 2 min., constantly stirring mixture. Transfer to original beaker with aid of 500 ml of H<sub>2</sub>O, stir, and allow to stand until supernatant liquid is clear. Draw off liquid by means of siphon of rubber tubing having 3 or 4 mm bore, using pinch clamp to control flow so that practically all liquid may be siphoned off. (Tilting beaker will also help to obtain this result.) If on standing a deposit forms, siphon again. Transfer hulls with aid of H<sub>2</sub>O to paper filter, wash several times with alcohol, and allow to dry to constant weight at room temp. When dry, carefully remove hulls from paper, using if necessary small stiff brush, and weigh. (Weighed Gooch crucible may be used instead of paper filter.) Multiply weight of hulls by 50 to obtain percentage of hulls in sample.

46

GRIT IN POULTRY AND SIMILAR FEEDS<sup>18</sup>—OFFICIAL, FIRST ACTION

Place 2 g of prepared sample, 1, thoroly mixed, in evaporating dish of ca 30 ml capacity. Add ca 5 ml of CHCl<sub>3</sub> and mix gently with glass rod until liquid comes in contact with all portions of sample. Brush particles adhering to rod into dish, and after pushing all particles down into the CHCl<sub>3</sub> with 25 mm circular or square cover-glass, use glass to skim off or pull floating portion of material over top of dish, taking care not to submerge cover-glass deep enough to disturb grit settled at bottom of dish. After skimming until surface of the CHCl<sub>3</sub> is nearly clear, slowly pour supernatant liquid into second evaporating dish. Wash sides of dish with a few ml more of CHCl<sub>3</sub> and repeat skimming and decanting operation until no floating particles remain (10–15 ml of CHCl<sub>3</sub>). When grit only remains, allow last traces of CHCl<sub>3</sub> to evaporate spontaneously, and weigh. Weight of residue  $\times 50$  = percentage of grit. After weighing examine residue for impurities. Also pour out CHCl<sub>3</sub> washings collected in second dish and observe whether any grit has been transferred to it during process.

47

BONE IN MEAT SCRAP OR TANKAGE<sup>18</sup>—OFFICIAL, FIRST ACTION

Separate bone as directed under 46. In some instances it may be found necessary, after first washing with CHCl<sub>3</sub>, to rub remaining residue of bone with glass rod or small pestle in order to bring some of adhering particles to surface of the CHCl<sub>3</sub>.

48

CALCIUM OXIDE IN MINERAL FEEDS<sup>18</sup>—OFFICIAL, FIRST ACTION

Weigh 2 g portion of finely ground sample into silica or porcelain dish and ignite in muffle to carbon-free ash, but avoid fusing. Boil residue in 40 ml of HCl (1+3)



and a few drops of  $\text{HNO}_3$ . Transfer to 250 ml volumetric flask, cool, dilute to mark, and mix thoroly. Pipet 25 ml of clear liquid into beaker, dilute to ca 100 ml, and add two drops of methyl red indicator. Add  $\text{NH}_4\text{OH}$  (1+1) dropwise to pH of 5.6, as shown by intermediate brownish color. If over-stepped, add with dropper  $\text{HCl}$  (1+3) to brownish point. Add 2 drops  $\text{HCl}$  (1+3). The color should now be pink (pH 2.5-3.0) instead of brown. Dilute to ca 150 ml, bring to boiling, and add slowly with constant stirring 10 ml of saturated (4.2%) soln of  $(\text{NH}_4)_2\text{C}_2\text{O}_4$ , which should also be hot. If red color changes to brown or yellow, add  $\text{HCl}$  (1+3) dropwise until color again changes to pink. Let stand overnight to allow precipitate to settle. Filter supernatant liquid thru quantitative filter paper on Gooch crucible, or on a fritted glass filter (Jena 1G4 is preferable), and wash precipitate thoroly with  $\text{NH}_4\text{OH}$  (1+50). Place filter paper or crucible with precipitate in original beaker, and add mixture of 125 ml of  $\text{H}_2\text{O}$  and 5 ml of  $\text{H}_2\text{SO}_4$ . Heat to  $70^\circ$  or above and titrate with 0.1  $N$   $\text{KMnO}_4$  until first slightly pink color is obtained. Presence of filter paper may cause the pink color to fade in few seconds. Correct for blank and calculate percentage of  $\text{CaO}$  in sample.

#### CYANOGENETIC GLUCOSIDES IN FEEDS AND SIMILAR MATERIALS<sup>49</sup>

49

##### *Qualitative Test—Official*

Prepare Na picrate paper by dipping strips into 1% soln of picric acid and drying, then dipping into 10% soln of  $\text{Na}_2\text{CO}_3$  and drying. Preserve these papers in stoppered bottle. Finely chop small quantity of plant material and place in test tube. Insert piece of the moist Na picrate paper in tube, taking care that it does not come in contact with material. Add a few drops of  $\text{CHCl}_3$  and stopper tube tightly. The Na picrate paper gradually turns orange, then brick red, if plant tissue contains cyanogenetic glucosides. (The test is delicate, and rapidity of change in color depends upon amount of free hydrocyanic acid present. This test works well with fresh plant materials, but in the case of relatively dry substances, particularly seeds of various plants, material should be ground and moistened with  $\text{H}_2\text{O}$  and allowed to hydrolyze in stoppered test tube containing Na picrate paper. If necessary, a small amount of emulsin may be added.)

#### HYDROCYANIC ACID FORMED BY HYDROLYSIS OF GLUCOSIDES IN BEANS<sup>50</sup>

50

##### *Acid Titration Method—Tentative*

Introduce 10-20 g of sample, ground to pass 20-mesh sieve, into 800 ml Kjeldahl flask, add 100 ml of  $\text{H}_2\text{O}$ , and macerate at room temp. for 2 hours. Add 100 ml of  $\text{H}_2\text{O}$  and distil with steam, collecting distillate in 20 ml of 0.02  $N$   $\text{AgNO}_3$  soln acidified with 1 ml of  $\text{HNO}_3$ . Before distilling, adjust apparatus so that tip of condenser dips below surface of liquid in receiver. When 150 ml has passed over, filter distillate thru Gooch crucible; wash receiver and Gooch with a little  $\text{H}_2\text{O}$ ; and titrate excess of  $\text{AgNO}_3$  in combined filtrate and washings with 0.02  $N$   $\text{KCNS}$  soln, using ferric alum indicator. 1 ml of 0.02  $N$   $\text{AgNO}_3$  soln = 0.54 mg of  $\text{HCN}$ .

51

##### *Alkaline Titration Method—Tentative*

Place 10-20 g of sample, ground to pass a 20-mesh sieve, in an 800 ml Kjeldahl flask, add ca 200 ml of  $\text{H}_2\text{O}$ , and allow to stand 2-4 hours. (The autolysis should be conducted with apparatus completely connected for distillation.) Distil with steam and collect 150-160 ml distillate in soln of  $\text{NaOH}$  (0.5 g in 20 ml of  $\text{H}_2\text{O}$ ).

To 100 ml of distillate (it is preferable to dilute to volume of 250 ml and titrate 100 ml aliquot) add 8 ml of 6  $N$   $\text{NH}_4\text{OH}$  and 2 ml of 5% soln of  $\text{KI}$  and titrate with

0.02 N  $\text{AgNO}_3$ , using micro buret. End point is faint but permanent turbidity and may be easily recognized, especially against black background. 1 ml of 0.02 N  $\text{AgNO}_3 = 1.08$  mg of HCN.

52

FERROUS SULFATE<sup>21</sup>—OFFICIAL, FIRST ACTION

Sift a portion of the feed thru a fine sieve (40-mesh) over sheet of white glazed paper whose entire surface has been moistened with soln of K ferricyanide (1+10) in such a manner that it will be distributed thinly over entire area. After few moments wash off feed under slow stream of  $\text{H}_2\text{O}$ . A blue speck or spot denotes particle of ferrous salt.

53

COPPER SULFATE<sup>21</sup>—OFFICIAL, FIRST ACTION

Proceed as directed under 52, except to use a soln of ferrocyanide (1+10). A brown speck or spot denotes particle of copper salt.

54

POTASSIUM IODIDE<sup>21</sup>—OFFICIAL, FIRST ACTION

So sift portion of feed over sheet of white glazed paper whose entire surface has been moistened with mixture of starch indicator and Br water (3 parts of former to 1 of latter) that the feed will be distributed thinly over entire area. A blue coloration denotes particle of an iodide. If an extremely small quantity of KI is to be detected, modify procedure by carefully charring 10 g or more of the feed, washing residue with small amount of  $\text{H}_2\text{O}$  and evaporating filtered soln in white evaporating dish so that the solids are concentrated on one small spot. When moistened with the starch indicator and Br water a blue coloration denotes presence of an iodide.

## IODINE IN MINERAL MIXED FEEDS

*Knapheide-Lamb Method<sup>22</sup>—Tentative*

55

## REAGENTS

(a) *Reduced phosphoric acid*.—20%. Reduce impurities in the  $\text{H}_3\text{PO}_4$  according to Kendall's method<sup>23</sup> by diluting the 85% acid with 4 volumes of  $\text{H}_2\text{O}$  and boiling for some time with Al strips.

(b) *Sodium thiosulfate soln*.—0.005 N. Preferably standardize as follows: Pipet into a beaker 25 ml of soln containing 0.1308 g of KI per liter and add 200 ml of  $\text{H}_2\text{O}$ , 5 ml of 20%  $\text{NaHSO}_3$  soln, and 2 or 3 g of NaOH. Neutralize mixture with sirupy  $\text{H}_3\text{PO}_4$ , add 1.0 ml in excess, and proceed as directed in regular determination. To calculate the mg of I to which 1 ml of the  $\text{Na}_2\text{S}_2\text{O}_3$  soln is equivalent, use

following formula: 
$$\frac{2.5}{\text{ml of Na}_2\text{S}_2\text{O}_3 \text{ soln}} \quad \text{(It is well to standardize the Na}_2\text{S}_2\text{O}_3 \text{ soln same day determination is made.)}$$

56

APPARATUS<sup>24</sup>

*Furnace*.—Use sheet-iron cylinder 4" in diameter and 12" high, having an opening in center of top large enough to accommodate 100 ml nickel crucible. Suspend  $2\frac{3}{4}$ " circular plate in center of cylinder 3" below top, for spreading flame, thereby preventing free flame from coming in contact with crucible, and providing uniform heat. Make slot at bottom of cylinder 1" wide by 3" high for admitting air and the burner tubing, and near top rim make eight  $\frac{1}{8}$ " holes to allow for escape of exhaust gases.

Fuse together in 100 ml nickel crucible 20 g of NaOH and 10 g of  $\text{KNO}_3$  and cool. Place evenly on top of fused alkali 1–10 g of sample (depending upon its composition and trouble experienced from frothing in fusion) and completely moisten with 5 ml of saturated NaOH soln and 10 ml of 80% alcohol. Place crucible on cold three-heat hot plate and evaporate alcohol at low heat. After 30 min. cautiously increase heat until crucible has been subjected to highest temp. of hot plate for 1½–2 hours. (Thorough heating at this stage prevents most of trouble from effervescence of material during fusion.) Then place crucible in furnace described previously or in similar furnace.

To prevent loss give close attention during fusion to mineral mixtures containing charcoal or organic matter because of violent reaction between the C and the  $\text{KNO}_3$ . If reaction becomes too violent, lift crucible from furnace for a moment, and if necessary cool bottom of crucible in beaker of  $\text{H}_2\text{O}$ . When mixture is in quiet state of fusion tip crucible on all sides in open flame to wash down fusion mixture. Add a few small crystals of  $\text{KNO}_3$  until no more gas is liberated by further additions, and again wash down sides of crucible in flame.

Pour melt out into clean crucible cover to cool, or turn crucible while cooling so that material solidifies on sides. Place cooled melt and crucible in 600 ml beaker, cover with  $\text{H}_2\text{O}$ , and heat below boiling point for a short time. After allowing mixture to stand overnight at room temp., rinse off crucible and cover and remove. In order to neutralize part of alkali and facilitate filtering, add 10 ml of sirupy  $\text{H}_3\text{PO}_4$  and place beaker on steam bath for 3–4 hours, stirring occasionally to break up mass and insure complete solution of the I. Cool beaker, filter off insoluble residue in 10 cm funnel and wash with cold  $\text{H}_2\text{O}$  into 800 ml beaker, adjusting volume to 550–600 ml. (The soln should be clear and colorless.)

In order to destroy nitrites, which interfere with titration with methyl orange, add 10 ml of 20%  $\text{NaHSO}_3$ , bring soln just to boiling point, and cool. Run ca 30 ml of 85%  $\text{H}_3\text{PO}_4$  in from buret, add a few drops of methyl orange soln, continue addition of  $\text{H}_3\text{PO}_4$  to neutral color of methyl orange, and finally add 1.5 ml of  $\text{H}_3\text{PO}_4$  in excess. (Total quantity of  $\text{H}_3\text{PO}_4$  required is generally not over 35 ml, except when presence of considerable C in sample has necessitated use of more  $\text{KNO}_3$ , which is mainly reduced to carbonate.) Use care not to run appreciably over end point, as excess acid gives low results. However, addition of the acid must be fairly rapid, as the color of methyl orange has tendency to fade, due to incomplete destruction of the nitrites.

After neutralization, add small lump of anthracite coal (0.5 cm in diameter) and boil soln at least 20 min., reducing volume to 400–500 ml. (Boiling is essential to remove all traces of sulfurous acid.) Again cool soln and add Br water until distinct and permanent yellow color is produced. Boil soln until colorless by reflected light and then for exactly 5 min. longer. Add a few crystals of salicylic acid to assure removal of last traces of Br, cool soln, and add 5 ml of 20% reduced  $\text{H}_3\text{PO}_4$  and 0.5–1.0 g of KI. Titrate soln in usual manner with 0.005 N  $\text{Na}_2\text{S}_2\text{O}_3$ , adding starch soln when brown color of liberated I is nearly gone. (The volume of the soln at final titration should be 400–500 ml.)

Place a sample that contains 3–4 mg of I in a 200–300 ml nickel dish. Add ca 5 g of  $\text{Na}_2\text{CO}_3$ , 5 ml of a saturated soln of NaOH, and 10 ml of alcohol, taking care that entire sample is moist. Dry at ca 100°, so that there will be no spattering upon subsequent heating (30 min. is usually sufficient).

Place dish and contents in muffle furnace, heated to 500°, and maintain at that

temp. 15 min. (Ignition of sample to 500° appears to be necessary only to carbonize any soluble organic matter that would be oxidized by Br water if not so treated. Temps. higher than 500° may be used if necessary.) After cooling add 25 ml of H<sub>2</sub>O, cover dish with watch-glass, and boil gently for 10 min. Transfer contents of dish to 18 cm filter paper and wash with boiling H<sub>2</sub>O, catching filtrate and washings in 600 ml beaker (soln should total ca 300 ml). Neutralize to methyl orange with 85% H<sub>3</sub>PO<sub>4</sub> and add 1 ml in excess.

Add excess of Br water and boil soln gently until colorless, and then 5 min. longer. Add a few crystals of salicylic acid and cool soln to ca 20°. Add 1 ml of 85% H<sub>3</sub>PO<sub>4</sub> and ca 0.5 g of KI and titrate I with 0.005 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> in usual way, using starch soln as indicator.

Standardize the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln by measuring into beaker exactly 25 ml of a soln containing 0.1308 g of KI per liter, adding 300 ml of H<sub>2</sub>O and 5 g of Na<sub>2</sub>CO<sub>3</sub>, neutralizing, and proceeding as directed above. (It is advisable to standardize on same day determinations are conducted.)

#### MANGANESE<sup>22</sup>—OFFICIAL, FIRST ACTION

59

##### REAGENT

*Standard potassium permanganate.*—Dissolve 1.4385 g of KMnO<sub>4</sub> by boiling with H<sub>2</sub>O. Dilute to 1 liter, let stand several days, and filter thru asbestos pad on Gooch crucible. Standardize with Na oxalate (soln should contain 500 p.p.m. of Mn). Transfer an aliquot containing 20 mg of Mn to a beaker. Add 100 ml of H<sub>2</sub>O, 15 ml of 85% H<sub>3</sub>PO<sub>4</sub>, and 0.3 g of KIO<sub>4</sub>, and bring to b.p. Cool, and dilute to 1 liter. Protect from light. Dilute this soln containing 20 p.p.m. of Mn with H<sub>2</sub>O that has been boiled with 0.3 g of KIO<sub>4</sub> per liter to make convenient working standards of known concentrations approximately like those to be compared.

60

##### DETERMINATION

Ash a 5 g sample at dull red heat in a porcelain dish. When cool, add 5 ml of H<sub>2</sub>SO<sub>4</sub> and 5 ml of HNO<sub>3</sub>. Evaporate to white fumes. If carbon is not completely destroyed, add further portions of HNO<sub>3</sub>, boiling after each addition. Cool slightly, transfer to 50 ml volumetric flask, and add 25 ml of H<sub>3</sub>PO<sub>4</sub> (8 ml of 85% H<sub>3</sub>PO<sub>4</sub> + 92 ml of H<sub>2</sub>O). Cool, make to volume, mix, and filter or let stand to allow insoluble matter to settle. Pipet 25 ml of clear soln into beaker or directly into 50 ml or 100 ml volumetric flask, and add 15 ml of H<sub>2</sub>O and 0.3 g of KIO<sub>4</sub> for each 15 mg of Mn present. Mix, and heat below b.p. 30 min., or until maximum color develops. Cool, and dilute with H<sub>2</sub>O to an accurately measured volume, usually 50 or 100 ml. Compare with the standard KMnO<sub>4</sub> soln in a colorimeter. Calculate p.p.m. of Mn in the sample.

#### CAROTENE<sup>27</sup>—TENTATIVE

61

##### EXTRACTION OF CAROTENE

Weigh out sample (1–5 g), transfer to 200 ml Erlenmeyer flask, and for each gram of sample add 20 ml of a freshly prepared, saturated soln of KOH in alcohol. Fit flask with reflux condenser, and boil contents on steam bath or hot plate 30 min. If portions of sample collect on sides of flask, wash down with alcohol from wash bottle. Cool contents of flask. (Volume of petroleum benzin to be used later for extraction may be reduced by filtering directly, after cooling, thru sintered glass funnel of No. 3 porosity, extracting residue with small portions of petroleum benzin until solvent is colorless and proceeding as directed below). Add 100 ml of Skellysolve (b.p. 60–70°), or petroleum benzin, and after shaking for minute or so and

allowing sediment to settle, decant Skellysolve-alcohol mixture into 500 ml separatory funnel. Repeat procedure twice more with 25 ml portions of Skellysolve, breaking up residue, which sometimes forms an adherent mass, by shaking with 10-15 ml of alcohol. After two or three additional extractions with 20 ml portions of Skellysolve (soln usually comes off colorless), discard residue.

Pour gently ca 100 ml of  $H_2O$  thru alcohol-Skellysolve soln in separatory funnel. Draw off the alkaline alcohol- $H_2O$  soln from bottom of funnel, and re-extract three times by shaking gently with 30 ml portions of Skellysolve, using two other separatory funnels. Combine Skellysolve extracts and wash with 50 ml portions of  $H_2O$  until free from alkali, as indicated by absence of color in wash  $H_2O$  when treated with phenolphthalein (ca 10 washings). (The use of larger amounts of  $H_2O$  (ca 100 ml) will reduce number of washings. Any small amount of alkali remaining will be removed by subsequent methyl alcohol and  $H_2O$  washings.)

Remove xanthophyl from the Skellysolve soln by extraction with 25 ml portions of 90% methyl alcohol (90 ml  $CH_3OH + 10$  ml  $H_2O$ ), shaking for 2 minutes. Continue these extractions until wash alcohol is colorless (6-12 washings, depending on amount of xanthophyl in sample). Wash the Skellysolve soln containing the carotene twice with 50 ml of  $H_2O$  to remove the alcohol, and adjust to volume (either dilution or concentration under reduced pressure) to obtain convenient concentration for measurement of the carotene. Filter into volumetric flask thru filter paper upon which is placed small amount of anhydrous  $Na_2SO_4$ . After making the carotene soln up to definite volume, determine concentration by spectrophotometer, photoelectric colorimeter, or colorimeter by comparison with 0.1% or 0.036%  $K_2Cr_2O_7$ .

## 62

## DETERMINATION

For each determination by spectrophotometric method make optical density measurements at wave lengths of 4500, 4700, and 4800 Å.U. Using the absorption coefficients calculated for beta carotene at these wave lengths, determine carotene concentration for each wave length, take average, and report results to 0.1 p.p.m.

## 63

*Extinction coefficients*

WAVE LENGTH, Å	SKELLYSOLVE B.P. 60-70°	PETROLEUM BENZIN B.P. 40-60°
4500	238	243
4550		231
4700	200	207
4800	212	212

Or, estimate amount of carotene in sample by comparing it colorimetrically against 0.1%  $K_2Cr_2O_7$ . Put the soln of the sample in left-hand cup of colorimeter and set scale at 0.5 cm, 1 cm, 2 cm, 3 cm, or 4 cm, according to amount of color present. Vary depth of dichromate soln in right-hand cup until density of color in both cups is equal, and make eight independent readings, recording them in mm. Average the readings. Make the dichromate readings between 4 mm and 12 mm on colorimeter. If necessary, make a reading below 4 mm, but repeat analysis with a larger sample.

By use of the table transform the depth in mm of 0.1% dichromate into p.p.m. of carotene. Then calculate the p.p.m. of carotene actually in sample (p), using following formula:

$$p = \frac{\text{p.p.m. of carotene (from table)} \times \text{ml of soln}}{\text{g of sample} \times \text{cm depth of sample soln}}$$

Report carotene to 0.1 p.p.m.

64

Table for calculating carotene

0.1% K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	CAROTENE	0.1% K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	CAROTENE
<i>mm</i>	<i>p.p.m.</i>	<i>mm</i>	<i>p.p.m.</i>
1.0	0.5	6.6	4.1
1.2	0.7	6.8	4.2
1.4	0.8	7.0	4.3
1.6	0.9	7.2	4.5
1.8	1.0	7.4	4.6
2.0	1.2	7.6	4.7
2.2	1.4	7.8	4.8
2.4	1.5	8.0	4.9
2.6	1.6	8.2	5.0
2.8	1.7	8.4	5.2
3.0	1.8	8.6	5.3
3.2	2.0	8.8	5.4
3.4	2.1	9.0	5.6
3.6	2.2	9.2	5.8
3.8	2.3	9.4	5.9
4.0	2.5	9.6	6.0
4.2	2.6	9.8	6.1
4.4	2.7	10.0	6.3
4.6	2.8	10.2	6.5
4.8	2.9	10.4	6.7
5.0	3.1	10.6	6.8
5.2	3.2	10.8	6.9
5.4	3.4	11.0	7.1
5.6	3.5	11.2	7.3
5.8	4.6	11.4	7.4
6.0	3.8	11.6	7.5
6.2	3.9	11.8	7.6
6.4	4.0	12.0	7.8

65

VITAMIN D FOR POULTRY<sup>28</sup>—TENTATIVE

(Applicable to fish and fish liver oils and their extracts, and to materials used for supplementing vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry.)

This assay is a comparison, under conditions specified below, of efficacy of the product under assay with that of U.S.P. Reference Cod Liver Oil in controlling ash content of bones of growing chicks.

The basal ration is a uniform mixture in proportions designated of following ingredients, which have been finely ground:

66

## BASIC RACHITIC RATION

	<i>per cent</i>
Ground yellow corn.....	58
Wheat flour middlings or wheat gray shorts.....	25
Crude domestic acid precipitated casein.....	12
Calcium phosphate (precipitated).....	2
Iodized salt (0.02% KI).....	1
Non-irradiated yeast (7% minimum N).....	2
To each kg of above mixture add 0.2 g of MnSO <sub>4</sub> ·4H <sub>2</sub> O.	

67

## PROCEDURE

Provide cages with screen bottoms and keep chicks away from sunshine or other source of actinic light that may influence calcification. Keep cages in rooms in which

wide variations in temp. are prevented (constant temp. preferred). Unless temp. of room is adequately controlled provide each cage with suitable electrical heating device. Start all birds to be used in one assay on same day and keep all conditions of environment uniform for all groups in the assay.

Make assay on groups of one- or two-day-old white Leghorn chicks as specified below. Provide for one or more negative control groups that receive no vitamin D, one or more positive control groups that receive U.S.P. Reference Cod Liver Oil, and one or more assay groups for each product to be assayed. Have positive control and assay groups consist of not less than 20 birds each, and the negative control group consist of not less than 10 birds. Make up rations for all groups in assay from one batch of basal ration. Add the Reference Cod Liver Oil to basal ration in such quantities as to produce measurable increase in percentage of bone ash above that obtained in negative control group (it is not possible to make comparisons if maximum bone ash is obtained). Add assay product to basal ration in such quantities as to permit direct comparison in response of assay and positive control groups. To basal ration of negative control group add corn oil equal in quantity to maximum quantity of oil fed to any group in assay and add corn oil to rations of other groups until total quantity of corn oil and oil containing vitamin D is equal to quantity of corn oil added to ration of negative control group. Feed chicks in respective groups prescribed ration and water (natural or distilled water) *ad libitum* for 21 days. Discard all chicks that weigh 100 g or less and all chicks that show abnormality or disease not related to vitamin D deficiency. At least 15 chicks must remain in each reference or assay group that is used in calculating the vitamin D potency of an assay product.

Kill chicks; remove left tibia of each bird and clean of adhering tissue. (To facilitate removal of adhering tissue bones may be placed in boiling H<sub>2</sub>O for not more than 2 min. The bones may be preserved in alcohol for extraction.) Completely extract bones with suitable fat solvent or solvents (20 hours with hot ethyl alcohol followed by 20 hours with ethyl ether is suitable, and the bones may be crushed to facilitate extraction). Dry extracted bones to constant weight in moisture oven, cool in desiccator, and weigh. Ash the moisture and fat-free bones from each group of birds in muffle furnace to constant weight at any given temp. between 450 and 550°, or if preferred for 1 hour at ca 850°. (Ash determination may be made on individual bones if desired.) Cool ash in desiccator and weigh. Use consistently throughout any one assay the specific procedure adopted for extraction, drying, and ashing of the bones.

68

## INTERPRETATION OF RESULTS

One A.O.A.C. chick unit of vitamin D is equal in biological activity for the chick to one unit of vitamin D in the U.S.P. Reference Cod Liver Oil in this method of assay. The product under assay meets its declared vitamin potency in A.O.A.C. chick units of vitamin D if percentage of ash in the moisture and fat-free bone produced in assay groups by given number of units of vitamin D is equal to or greater than percentage of ash produced by same number of units of vitamin D from the U.S.P. Reference Cod Liver Oil.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 8, 295 (1925); 9, 30 (1926); 17, 68 (1934).
- <sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull., 122, p. 219; 132, p. 150.
- <sup>3</sup> J. Assoc. Official Agr. Chem., 13, 40 (1930); 15, 27 (1932); 17, 68 (1934); 18, 80 (1935).
- <sup>4</sup> *Ibid.*, 18, 81, 369 (1935); 19, 94 (1936); 21, 86 (1938).
- <sup>5</sup> *Ibid.*, 17, 190 (1934); 18, 80 (1935).

- <sup>6</sup> Ibid., 7, 339 (1924).  
<sup>7</sup> U. S. Dept. Agr. Bur. Chem. Circ., 71; J. Assoc. Official Agr. Chem., 18, 80 (1935).  
<sup>8</sup> J. Am. Chem. Soc., 26, 266 (1904).  
<sup>9</sup> J. Agr. Research, 23, 995 (1923); J. Assoc. Official Agr. Chem., 9, 31 (1926).  
<sup>10</sup> J. Assoc. Official Agr. Chem., 21, 596 (1938).  
<sup>11</sup> J. Landw., 48, 357 (1900); 49, 7 (1901).  
<sup>12</sup> U. S. Dept. Agr. Bur. Chem. Bull., 73, p. 173.  
<sup>13</sup> Ibid., 137, p. 152.  
<sup>14</sup> J. Assoc. Official Agr. Chemists, 22, 526 (1939); 23, 82 (1940).  
<sup>15</sup> Ibid., 9, 32 (1926); 19, 95 (1936).  
<sup>16</sup> Ibid., 5, 77 (1921).  
<sup>17</sup> Ibid., 9, 149 (1926); 10, 32 (1927).  
<sup>18</sup> Ibid., 5, 424 (1922).  
<sup>19</sup> Ibid., 10, 177 (1927); 19, 94 (1936).  
<sup>20</sup> J. Am. Chem. Soc., 37, 601 (1915); J. Assoc. Official Agr. Chem., 4, 151 (1920); 17, 69 (1934); 18, 348 (1935); 19, 94 (1936).  
<sup>21</sup> J. Assoc. Official Agr. Chem., 14, 142 (1931); 15, 77 (1932).  
<sup>22</sup> Ibid., 17, 67 (1934); 18, 80 (1935); J. Am. Chem. Soc., 50, 2121 (1928).  
<sup>23</sup> J. Biol. Chem., 43, 150 (1920).  
<sup>24</sup> J. Assoc. Official Agr. Chem., 14, 150 (1931).  
<sup>25</sup> J. Official Agr. Chem., 18, 338 (1935); 21, 86 (1938).  
<sup>26</sup> Ibid., 22, 78, 673 (1939); J. Am. Chem. Soc., 39, 2366 (1917); G. Frederick Smith Chemical Co. Pubs., 1, 2nd ed., August (1933).  
<sup>27</sup> J. Assoc. Official Agr. Chem., 20, 464 (1937); 22, 79 (1939).  
<sup>28</sup> Ibid., 22, 81 (1939).



## XXVIII. MEAT AND MEAT PRODUCTS

### MEAT

1

#### PREPARATION OF SAMPLE—OFFICIAL

To prevent loss of  $H_2O$  during preparation and subsequent handling do not use small samples. Keep the ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in following manner:

(a) *Fresh meats, dried meats, cured meats, smoked meats, etc.*—Separate as completely as possible from any bone; pass rapidly thru food chopper 3 times, thoroly mixing after each grinding; and begin all determinations as soon as practicable. If any delay occurs, chill sample to inhibit decomposition.

(b) *Canned meats.*—Pass entire contents of can thru food chopper, as directed under (a).

(c) *Sausages.*—Remove from casings and pass thru food chopper, as directed under (a).

Dry the portions of the samples under (a), (b), and (c) not needed for immediate analysis, either in vacuo below  $60^\circ$  or by evaporating on steam bath 2 or 3 times with alcohol. Extract fat from dried product with gasoline (b.p. below  $60^\circ$ ) and allow gasoline to evaporate spontaneously, finally expelling last traces by heating for short time on steam bath. Do not heat sample or separated fat longer than necessary because of tendency to decompose. Reserve fat for examination as directed under XXXI, keeping it in cool place, and complete examination before it becomes rancid.

2

#### MOISTURE—OFFICIAL

Proceed as directed under XXVII, 2 or 6, following 6 when dried sample is to be used for further determinations.

3

#### ADDED WATER IN SAUSAGE—TENTATIVE

(a) *Moisture.*—Weigh accurately ca 10 g of the ground sample into tared weighing bottle, ca 2" in diameter, containing short glass rod flattened at one end. Remove 2.5–3 g for protein determination. Reweigh remainder in bottle, spreading it out in thin layer over sides and bottom by means of the glass rod, and use this sample for determination of moisture. Dry in air at atmospheric pressure at temp. of  $101\text{--}102^\circ$  16–18 hours, or at temp. of ca  $125^\circ$  (not lower than  $120^\circ$  nor higher than  $130^\circ$ ) 2–3 hours, or until no significant loss of weight occurs on subsequent drying for period of 1–2 hours. If preferred, determine moisture as directed under 2.

(b) *Nitrogen.*—Determine total N as directed under II, 21, 22, or 23. Protein = total N  $\times 6.25$ .

(c) *Added water.*—Multiply percentage of protein calculated from N determination (b) by 4 and subtract result from percentage of moisture found. Report difference, if any, as added  $H_2O$ .

4

#### ASH—OFFICIAL.—See XXXIV, 9 or 10.

5

#### SALT<sup>2</sup>—OFFICIAL

Moisten  $2\frac{1}{2}$ –3 g of the finely comminuted and thoroly mixed sample in Pt dish with 20 ml of 5%  $Na_2CO_3$  soln, evaporate to dryness, and ignite at temp. not exceeding dull redness. Extract with hot  $H_2O$ , filter, and wash. Return residue to dish and ignite to an ash. Dissolve ash in  $HNO_3$  (1 + 4), filter to free from any insoluble residue, wash thoroly, and add wash soln to the  $H_2O$  extract. Determine Cl in combined filtrate and washings as directed in XII, 37.

6 CRUDE FAT OR ETHER EXTRACT—OFFICIAL.—See XXVII, 22

7 TOTAL PHOSPHORUS—OFFICIAL

Destroy organic matter as directed under II, 8(c) or (d), and proceed as directed under II, 9 or 12.

8 TOTAL NITROGEN<sup>2</sup>—OFFICIAL

Proceed as directed under II, 21, 22, or 23, using ca 2 g of the fresh sample.

#### AMMONIA

##### *Aeration Method<sup>1</sup>—Tentative*

9

#### APPARATUS

Use apparatus illustrated in Fig. 34. *A* is wash bottle  $\frac{1}{4}$  full of  $H_2SO_4$  (1+9); *B* is tube containing sample; *C* is rubber disk; and *D* is 5 ml bulb to prevent spray from being carried over into tube *E*, which contains the standard acid; *F* is safety bottle.

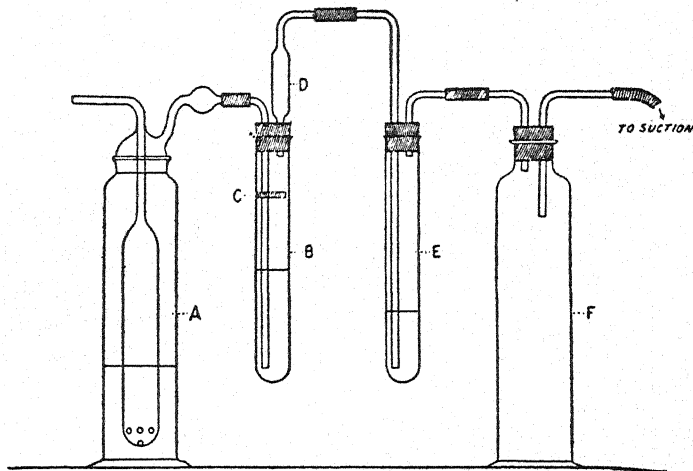


FIG. 34.—APPARATUS FOR DETERMINATION OF AMMONIA

10

#### DETERMINATION

Introduce 2–4 g of the finely divided meat into tube *B* and add 20 ml of  $NH_3$ -free  $H_2O$ . Place measured quantity of 0.04 *N* or 0.02 *N*  $H_2SO_4$  or  $HCl$  in tube *E*. Add 1 ml of saturated *K* oxalate soln to sample in tube *B*, introduce a few drops of kerosene, and finally add just sufficient saturated *Na* or *K* carbonate soln to render mixture alkaline. Place tubes in position at once, pass air thru apparatus, and titrate the standard acid in tube *E* at hourly intervals until  $NH_3$  ceases to be given off, using methyl red, cochineal, or congo red indicator. (If preferred, the  $NH_3$  collected in tube *E* may be determined by nesslerizing as directed under XXXVII, 11.)

#### NITRATES (INCLUDING ALSO NITRITES)

##### *Ferrous Chloride Method<sup>2</sup>—Tentative*

11

#### REAGENTS

(a) *Ferrous chloride soln.*—Dissolve 400 g of nails, tacks, or other small pieces of

iron in 2 liter Florence flask with 1 liter of HCl, excluding air from flask by means of stopper equipped with Bunsen valve. When evolution of gas ceases, transfer, and keep the soln in completely filled 50 ml glass-stoppered bottles. Use only freshly opened bottles of the reagent.

(b) *Standard sodium nitrate soln.*—Dissolve 2 g of  $\text{NaNO}_3$  in 1 liter of recently boiled  $\text{H}_2\text{O}$ . Determine NO in 50 ml of this soln (equivalent to 0.1 g of  $\text{NaNO}_3$ ) as directed under 13.

## 12

## APPARATUS

Clamp to an iron stand a 500 ml Kjeldahl flask fitted with 2-holed stopper. Thru one of holes pass stem of 100–125 ml cylindrical separatory funnel having a glass stopcock, and into other fit delivery tube leading downward at an angle from flask into trough containing a soln of commercial NaOH (1+1). Terminate upper end of delivery tube just below stopper in flask and place lower end, which is slightly constricted, bent upward, and covered with rubber tubing to prevent fracture, under surface of the NaOH soln in trough, the exit being just below mouth of an inverted measuring tube (50 ml plain eudiometer tube) filled with the soln of NaOH. A single coil of tin tubing fitted into trough and carrying current of cold  $\text{H}_2\text{O}$  greatly facilitates determination.

## 13

## DETERMINATION

Extract 100 g of the sample by boiling 6–7 times with successive 35–50 ml portions of  $\text{H}_2\text{O}$ , decant extracts thru muslin or paper filter into casserole, and evaporate combined extracts to volume of ca 50 ml. Introduce 50 ml of the  $\text{FeCl}_2$  soln and 50 ml of HCl (1+2.5) into Kjeldahl flask, close stopcock of funnel, move end of delivery tube so that escaping air will not pass into measuring tube, and boil contents of flask until the air is completely expelled. Place exit end of delivery tube beneath measuring tube and boil the contents of the flask 1 min. longer to make certain that no air remains. Introduce 50 ml of standard  $\text{NaNO}_3$  soln into flask, a little at a time thru funnel, continuously boiling contents of flask to force the NO gas into measuring tube. Finally rinse funnel 3 or 4 times with 5–10 ml of recently boiled  $\text{H}_2\text{O}$ , adding rinsings to contents of evolution flask in manner described above. When evolution of gas ceases, cover opening of measuring tube with a porcelain crucible, using tongs, and carefully transfer tube to tall glass jar containing a soln of NaOH (1+1), kept at room temp. The temp. of the surrounding caustic soln will soon (10–15 min.) be imparted to contents of the tube, and the volume of NO is read with tube in such a position that level of soln within tube coincides with level outside. Calculate percentage of nitrates and nitrites as  $\text{NaNO}_3$  from volume of NO obtained from sample compared with volume obtained from 0.1 g of  $\text{NaNO}_3$ , both measured under identical conditions.

After measuring tube has been removed, quickly insert over the delivery tube another tube filled with soln of commercial NaOH (1+1) and boil 1 min. longer to make sure that all the NO has been expelled. Run another 50 ml portion of standard soln into apparatus and repeat determination. Then run sample in same manner, making certain that all the NO gas has been expelled and rinsing out both casserole and funnel 3 or 4 times; 6 to 8 determinations may be made, excluding 2 standards. Finally run another standard. The 3 standards should check within 0.5 ml on 30–35 ml; 0.1 g of  $\text{NaNO}_3$  should give 26.36 ml of NO at  $0^\circ$  and 760 mm pressure. Report results as percentage of  $\text{NaNO}_3$ .

*Xylenol Method<sup>a</sup>—Tentative*

14

## APPARATUS

Use simple distillation apparatus, including distillation bulb. A glass condenser of a type utilizing a thin, rapidly moving film of  $H_2O$  as cooling medium (West type) is recommended. Quickly remove any nitro-xylenol solidifying in condenser by stopping flow of  $H_2O$  and allowing condenser to become warm.

15

## REAGENTS

(a) *Meta-xylenol*.—1-hydroxy, 2,4-dimethylbenzene. Eastman's preparation No. 1150, or equivalent.

(b) *Silver ammonium hydroxide*.—Dissolve 5 g of nitrate-free  $Ag_2SO_4$  in 60 ml of  $NH_4OH$ . Heat mixture to boiling, concentrate to ca 30 ml, cool, and dilute to 100 ml with  $H_2O$ .

(c) *Bromocresol green indicator*.—Dissolve 0.1 g of bromocresol green in 1.5 ml of 0.1 N NaOH, and make up to 100 ml with  $H_2O$ .

(d) *Standard nitrate soln*.—Dissolve 0.1804 g of recrystallized  $KNO_3$  in  $H_2O$  and make up to 1 liter, or dilute 17.85 ml of 0.1 N  $HNO_3$  to 1 liter. 10 ml contains 0.25 mg of nitrate nitrogen.

16

## DETERMINATION

Mix 5–10 g of the finely comminuted and thoroly mixed sample with 80 ml of warm  $H_2O$ . Break up all lumps and heat on steam bath for 1 hour, stirring occasionally. Transfer to 100 ml volumetric flask, cool, make up to mark, and mix. Filter, or allow to settle, and pipet 40 ml of filtrate, or supernatant liquid, into 50 ml volumetric flask. (No correction for volume occupied by the meat is necessary.) Add 3 drops of the bromocresol green indicator. Add  $H_2SO_4$  (1+10) dropwise until color changes to yellow. Oxidize nitrites to nitrates by adding 0.2 N  $KMnO_4$  soln dropwise with shaking until faint pink color remains 15–30 seconds. Add 1 ml of  $H_2SO_4$  (1+10) and 1 ml of phosphotungstic acid soln (20 g in 100 ml). Make up to mark, mix, and filter.

Measure into 500 ml flask (Erlenmeyer is satisfactory) an aliquot (not more than 20 ml) containing from 0.005 to 0.15 mg of nitrate nitrogen. (If more than 20 ml is required, make slightly alkaline and concentrate by evaporation.) Add sufficient quantity of the silver ammonium hydroxide soln to precipitate all chlorides and most of excess phosphotungstic acid. (A slight excess of the silver reagent is not harmful; 1 or 2 ml is usually sufficient.) Without decanting or filtering, add volume of  $H_2SO_4$  (3+1) ca three times volume of liquid in flask. Stopper flask, mix, cool to ca 35°, add 0.05 ml (1–2 drops) of the m-xylenol, stopper, shake, and hold at 30–40° for 30 min.

(A yellow to brownish yellow color, indicative of nitrates, will appear. A bright red precipitate, due to incomplete removal of phosphotungstic acid, may also appear. A slight excess of phosphotungstic acid causes no interference but a large excess may do so.)

After nitration is complete, add 150 ml of  $H_2O$ , taking care to wash off stopper, and distil until 40–50 ml has passed over into receiver containing 5 ml of NaOH (10 g per liter). Transfer distillate to 100 ml volumetric flask, make up to volume with  $H_2O$ , and determine nitrate nitrogen by comparing color of suitable aliquot with set of graded color standards containing 0.003–0.006 mg of nitrate nitrogen.

Prepare the color standard from 10 ml of the nitrate standard as directed previ-

ously, using 0.05 ml of the m-xyleneol and 30 ml of  $\text{H}_2\text{SO}_4$  (3+1), and making up distillate to 500 ml. Prepare color standard fresh each day, as it becomes cloudy on standing.

17

NITRITES<sup>1</sup>—TENTATIVE

(Applicable to cured meats.)

Weigh 5 g of finely comminuted and thoroly mixed sample into 50 ml beaker. Add ca 40 ml of nitrite-free  $\text{H}_2\text{O}$  heated to temp. of  $80^\circ$ . Mix thoroly by stirring with glass rod, taking care to break up all lumps, and transfer to 500 ml graduated flask. Wash out beaker and rod thoroly with successive portions of the hot  $\text{H}_2\text{O}$ , adding all washings to flask. Add sufficient hot  $\text{H}_2\text{O}$  to bring contents of flask to volume of ca 300 ml, transfer flask to steam bath, and let stand 2 hours, shaking occasionally. Add 5 ml of saturated  $\text{HgCl}_2$  soln and mix. Cool to room temp., make up to mark with nitrite-free  $\text{H}_2\text{O}$ , and mix again. Filter, and determine nitrite N in suitable aliquot as directed under XXXVII, 15, reporting results as parts of  $\text{NaNO}_2$  per million.

## STARCH

(In chopped meat, sausage, deviled meat, etc.)

18

*Qualitative Test—Tentative*

Treat 5–6 g of the sample with boiling  $\text{H}_2\text{O}$  for 2–3 min., cool mixture, and test supernatant liquid with I soln, XXXIII, 29(f). (In interpreting this test it should be remembered that a small quantity of starch may be present as result of use of spices. If marked reaction is given, however, it may be concluded that starch or flour has been added, and a quantitative determination should be made. The qualitative test may be replaced by microscopic examination, which discloses not only presence of added starch but also variety used.)

19

*Quantitative Method<sup>3</sup>—Tentative*

Treat in 200 ml beaker 10 g of the finely divided sample with 75 ml of an 8% soln of KOH in 95% alcohol and heat on steam bath until all meat is dissolved (30–45 min.). Add equal volume of 95% alcohol, cool, and allow to stand at least an hour. Filter by suction thru thin layer of asbestos in Gooch crucible. Wash twice with warm 4% soln of KOH in alcohol, 50% by volume, and then twice with warm 50% alcohol. Discard washings. Retain as much of precipitate in beaker as possible until last washing. Place crucible with contents in the original beaker and add 40 ml of  $\text{H}_2\text{O}$  and 25 ml of  $\text{H}_2\text{SO}_4$ . Stir during addition of acid and make sure that it comes in contact with all the precipitate. Allow to stand ca 5 min., add 40 ml of  $\text{H}_2\text{O}$ , and heat just to boiling, stirring constantly. Transfer soln to 250 ml volumetric flask, add 2 ml of 20% phosphotungstic acid soln, allow to cool to room temp., and make up to mark with  $\text{H}_2\text{O}$ . Filter thru starch-free filter paper, pipet 100 ml of filtrate into 200 ml volumetric flask, neutralize with NaOH (1+1), make up to volume, and determine dextrose present in 50 ml portion of filtrate as directed under XXXIV, 38, titrating the  $\text{Cu}_2\text{O}$  precipitate as directed under XXXIV, 41. Weight of dextrose  $\times 0.9$  = weight of starch.

## GLYCOGEN

20

*Qualitative Test<sup>3</sup>—Tentative*

Boil 50 g of the macerated sample with 50 ml of  $\text{H}_2\text{O}$  for 15–30 min. Filter broth thru moistened filter paper or fine linen. To portion of filtrate in test tube add a

few drops of a mixture of 2 parts of I, 4 parts of KI, and 100 parts of  $\text{H}_2\text{O}$ . If a considerable quantity of glycogen is present, it produces a dark brown color; this color is destroyed by heating, but it reappears on cooling. If starch is present, it may be precipitated by treating the water extract with two volumes of glacial acetic acid and after filtering applying test for glycogen to filtrate.

*Quantitative Method<sup>10</sup>—Tentative*

21

PREPARATION OF SOLUTION

Weigh by difference ca 25 g of the finely ground and thoroly mixed sample. Place in 400 ml beaker and mix with 50 ml of KOH soln (1.5+1), free from carbonate. Cover beaker with watch-glass and digest on steam bath for 2 hours, stirring occasionally. Dilute to ca 200 ml with cold  $\text{H}_2\text{O}$ .

22

DETERMINATION

Add to the soln, 21, an equal volume of 95% alcohol, cover with watch-glass, and set aside for 10–12 hours. Decant supernatant liquid thru folded 18.5 cm filter, allowing glycogen to remain in beaker, and wash by decantation with 66% alcohol (2 volumes 95% alcohol +1 of  $\text{H}_2\text{O}$ ) until glycogen is white, or nearly so. (Usually ca 4 washings are required.) Transfer washed precipitate from beaker to filter and wash 2 or 3 times with the 66% alcohol. (Soln filters slowly, and funnel should be covered with watch-glass to prevent excessive evaporation. The albuminous substance present retards filtration if it is permitted to dry on the paper. If washing by decantation is not made as complete as possible, it will be difficult to obtain the glycogen free from the coloring matter.)

After washing is completed, close bottom of funnel by piece of rubber tubing and pinch-cock. Fill funnel with warm  $\text{H}_2\text{O}$ , cover with watch-glass, and let stand 2–3 hours, or overnight. Open pinch-cock and allow all the soln to pass thru filter into beaker. Close funnel with the pinch-cock and fill with warm  $\text{H}_2\text{O}$  as before. Allow this  $\text{H}_2\text{O}$  to remain in funnel for 1 hour and then filter as before. At first the glycogen soln appears quite turbid. Continue washing with warm  $\text{H}_2\text{O}$  until filtrate becomes perfectly clear. To the soln of glycogen in  $\text{H}_2\text{O}$ , add double its volume of 95% alcohol and let stand overnight to complete the reprecipitation of the glycogen. Filter, and wash as before with 66% alcohol.

If desired, the last filtration may be made thru a weighed Gooch crucible and the weight of glycogen determined after drying to constant weight. This gives results that are approximately correct. More satisfactory results are obtained by hydrolyzing the glycogen with HCl (1+3) and determining the resultant dextrose. Dissolve the glycogen on filter in warm  $\text{H}_2\text{O}$  as directed above, collecting filtrate and washings in 300 ml volumetric flask and keeping volume within 225 ml. Add 12.5 ml of HCl to combined filtrate and washings, mix, and place in boiling water bath for 3 hours. Cool, neutralize with 10% NaOH soln, cool again, make up to volume with  $\text{H}_2\text{O}$ , and determine dextrose in aliquot of the soln as directed under XXXIV, 38, determining reduced Cu as directed under XXXIV, 41. Corresponding weight of dextrose  $\times 0.9$  = its equivalent of glycogen. Correct this result for dilution to obtain percentage of glycogen in sample.

23

SUGAR—TENTATIVE

Weigh 100 g of the finely ground sample into 600 ml beaker, add 200 ml of  $\text{H}_2\text{O}$ , heat to boiling, and boil gently for 5 min. Stir contents of beaker frequently during this and subsequent extractions to prevent bumping. (When several samples are extracted at same time a mechanical stirring device is practically a necessity.)

Remove beaker from flame, allow insoluble matter to settle, and decant clear liquid on asbestos mat in a 4" funnel. Filter with aid of suction. Add 150 ml of hot  $H_2O$  to residue in beaker, boil gently for 5 min., let settle, and decant clear liquid as directed previously. Repeat operation, finally transfer contents of beaker to funnel, wash with 150–200 ml of hot  $H_2O$ , and press meat residue as dry as possible. Transfer contents of filter flask to evaporating dish and evaporate on steam bath to volume of ca 25 ml but not to dryness. Transfer extract to 100 ml volumetric flask, taking care that volume of liquid does not exceed 60 ml. Add 25–35 ml of phosphotungstic acid soln (1+1), shake vigorously, let stand a few minutes for gas bubbles to rise to surface, make to volume, shake, and either filter or centrifuge. (Use of centrifuge is to be preferred, because a larger volume of liquid is obtained.) Test a portion of filtrate with dry phosphotungstic acid for complete precipitation. If appreciable precipitate forms, take an aliquot of filtrate, add 5–10 ml of the phosphotungstic acid soln, make to volume, filter, and test filtrate for complete precipitation. Filtrate should also show not more than slight reaction for creatinin when tested by adding to 5 ml a few drops of saturated aqueous soln of picric acid and making mixture alkaline with a few drops of 10% NaOH soln.<sup>11</sup>

Transfer 50 ml of clarified extract to 100 ml volumetric flask, add 5 ml of HCl, and invert soln as directed under XXVII, 30. Cool soln, neutralize to litmus, cool, make to volume, and filter. To filtrate add sufficient dry powdered KCl to precipitate excess phosphotungstic acid, filter, test filtrate for complete precipitation, and determine reducing sugar as directed under XXXIV, 34 or 38, ascertaining quantity of reduced Cu as directed under XXXIV, 43. Calculate total sugar as dextrose.

If an abnormal reduction is obtained when clarified meat extract is boiled with Fehling's soln, i.e., if soln turns yellow, brown, green, or muddy in appearance instead of reddish-blue, discard determination, since incomplete precipitation of nitrogenous compounds, due to use of insufficient phosphotungstic acid, is indicated.

24 PRESERVATIVES—OFFICIAL.—See XXXII.

25 METALS—TENTATIVE.—See XXIX.

26 COLORING MATTERS—TENTATIVE.—See XXI.

#### SOLUBLE AND INSOLUBLE NITROGEN—TENTATIVE

27 PREPARATION OF SOLUTION

Exhaust 7–25 g of the sample (depending upon  $H_2O$  content) in following manner: Weigh into 150 ml beaker, add 5–10 ml of cold (15°)  $NH_3$ -free  $H_2O$ , and stir to homogeneous paste. Add 50 ml of cold  $H_2O$ , stir for 15 min. at 3 min. intervals, let stand for 2–3 min., and decant liquid thru quantitative filter, collecting filtrate in 500 ml volumetric flask. Drain beaker, pressing out liquid from meat residue by aid of glass rod. Add to residue in beaker 50 ml of cold  $H_2O$ , stir for 5 min., allow to stand 2–3 min., and decant as before. If a considerable portion of meat is transferred to filter, return it to beaker by means of glass rod. Repeat extractions, using two 50 ml portions and four 25 ml portions of cold  $H_2O$ . After the last extraction transfer entire insoluble portion to filter and wash with three 10 ml portions of  $H_2O$ , allowing material to drain thoroly after each addition of  $H_2O$ . Dilute to mark and mix thoroly.

28 DETERMINATION

Determine total N in 50 ml aliquot of soln obtained, 27, proceeding as directed under II, 21, 22, or 23. Total N – soluble N = insoluble N.

29

## COAGULABLE NITROGEN—TENTATIVE

(For uncooked meat only.)

Measure 150 ml of extract, 27, into 250 ml beaker and evaporate to 40 ml on steam bath, stirring occasionally. Neutralize to phenolphthalein, using the indicator outside the soln to avoid subsequent interference in the determination of creatin, 32. Add 1 ml of 0.1 N acetic acid, and boil gently for 5 min. (Coagulum should separate at once, leaving clear liquid.) Filter thru quantitative paper and wash beaker thoroly 4 times with hot H<sub>2</sub>O, taking special care to clean sides. Finally wash coagulum on filter 3 times, dilute combined filtrate and washings to definite volume, and reserve for determination of proteose, peptone, and gelatin, 30, and creatin, 32. Transfer coagulum with paper to Kjeldahl flask and remove, with H<sub>2</sub>SO<sub>4</sub>, any of material adhering to beaker, taking the usual 25 ml of acid in 5 ml portions for this purpose, heating acid in beaker on hot plate, and rubbing with glass rod. Proceed as directed under 8.

## PROTEOSE, PEPTONE, AND GELATIN NITROGEN

30

*Modified Tannin-Salt Method<sup>12</sup>—Tentative*

Transfer 50 ml aliquot of filtrate, 29, to 100 ml volumetric flask, add 15 g of NaCl and 10 ml of cold H<sub>2</sub>O, shake until the NaCl has dissolved, and cool to 12°. Add 30 ml of 24% tannin soln cooled to 12°, dilute to mark with H<sub>2</sub>O previously cooled to 12°, shake, and allow mixture to stand at temp. of 12° for 12 hours, or overnight. Filter at 12°, transfer 50 ml of filtrate to Kjeldahl flask, and add a few drops of H<sub>2</sub>SO<sub>4</sub>. Place flask in steam bath, connect with vacuum pump, and evaporate to dryness. Determine N in residue as directed under II, 21, using 30 ml of H<sub>2</sub>SO<sub>4</sub> for the digestion. Conduct blank determination, using same quantity of reagents, and correct result accordingly. Multiply corrected result by 2 and deduct quantity of N found from N determined in another 50 ml aliquot of filtrate, 29, that has not been treated with tannin-salt. Difference  $\times 6.25$  = percentage of proteose, peptone, and gelatin.

31

## MEAT BASES—TENTATIVE

Deduct from percentage of total N, 8, the sum of percentages of N obtained in determination of insoluble N, 28, coagulable N, 29, and proteose, peptone, and gelatin, 30, to obtain percentage of N of meat bases. Multiply result by 3.12 to obtain percentage of meat bases.

32

## CREATIN—OFFICIAL

Evaporate aliquot or remaining portion of filtrate and washings from coagulable N, 29 (a portion having been used in 30) to 5–10 ml; transfer with minimum quantity of hot H<sub>2</sub>O to 50 ml volumetric flask, keeping volume below 30 ml; add 10 ml of 2 N HCl; and mix. Hydrolyze in autoclave at 117–120° for 20 min., allow flask to cool somewhat, remove, and chill under running H<sub>2</sub>O. Partially neutralize excess of acid by adding 7.5 ml of 10% NaOH soln, free from carbonates, dilute to mark, and mix. Make preliminary reading on 20 ml with Duboscq colorimeter to ascertain volume to use to obtain a reading of ca 8 mm. Transfer such a volume of the soln to a 500 ml volumetric flask and add 10 ml of 10% NaOH and 30 ml of saturated picric acid soln (1.2%). Mix, rotate 30 seconds, and let stand exactly 4.5 min. Dilute to mark at once with H<sub>2</sub>O, shake thoroly, and compare, preferably in a Duboscq colorimeter, with a standard soln prepared by treating 50 ml of a soln of creatinin



zinc chloride in 0.1 *N* HCl containing 1.603 g per liter (0.001 g creatinin per ml), with NaOH and picric acid, and making up to 500 ml in manner described above.

### AMINO NITROGEN

#### *Van Slyke Method*<sup>13</sup>—*Tentative*

33

#### APPARATUS

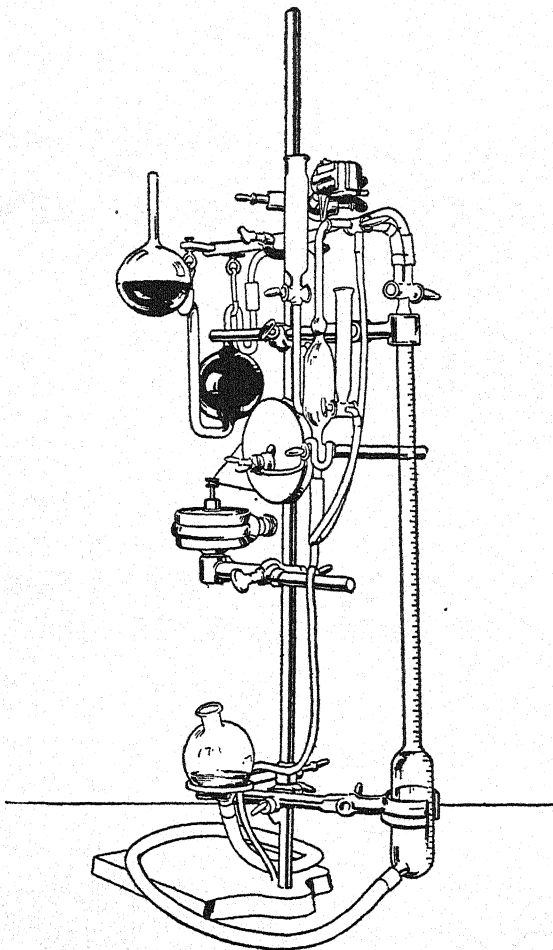


FIG. 35.—VAN SLYKE APPARATUS FOR DETERMINATION OF AMINO NITROGEN

shake apparatus rapidly with motor for 2 min., these operations being for purpose of expelling all air from *D*. Turn *c* and *f* so that *D* and *F* are connected.

Measure off in *B* 10 ml or less, as case may be, of the soln of the sample containing not more than 20 mg of amino N (ca 1–2 g of the sample in the case of meat extracts) and allow it to run into *D*. Connect *D* with motor as shown in Fig. 35 and shake for 5 min.

Use apparatus shown in Figs. 35 and 36, the former illustrating manner in which entire apparatus is arranged and the latter showing details of the deaminizing bulb and connections. The Hempel gas pipet is filled with a soln containing 50 g of  $\text{KMnO}_4$  and 25 g of KOH per liter.

#### 34 DETERMINATION

Fill with  $\text{H}_2\text{O}$  buret (*F*), capillary tube leading to Hempel pipet, and also other capillary as far as *c*. Introduce into *A* sufficient glacial acetic acid to fill  $\frac{1}{4}$  of *D*, etching tube *A* with mark to measure this quantity. Allow acid to run into *D*, and turn cock *c* so as to allow air to escape from *D*. Pour  $\text{NaNO}_2$  soln (300 g per liter) into *A* until *D* is filled and enough excess is present to rise a little above the cock into *A*. *A* is also marked for measuring off this quantity. Close gas exit from *D* at *c*, and, *a* being open, shake *D* a few seconds until liquid is forced down to 20 ml mark in *D*. Close *a*, open *c*, and

If the soln of sample is viscous and threatens to foam over, rinse out *B*, and thru it introduce a little caprylic alcohol into *D*, or if it is known beforehand that sample will cause excessive foaming, introduce a little caprylic alcohol into *D* thru *B*, rinsing *B* with alcohol and ether or drying with roll of filter paper before adding soln of sample.

During the shaking there is evolution of *N* mixed with *NO*, the gases being collected in *F*. Force all the gas in *D* into *F* by opening *a* and filling *D* with liquid from *A*. Connect *F* with Hempel pipet and force the gas into latter by means of leveling bulb, allowing cock *a* to remain open during this and succeeding operation in order to permit displacement of the liquid in *D* by the *NO* formed in interval. Connect driving rod with pipet by lifting hook from shoulder of *D* and placing other hook, on opposite side of driving rod, over horizontal lower tube of pipet. Shaking pipet rather slowly for a few minutes completes absorption of *NO* except with almost completely exhausted permanganate solns. Return gas to buret and adjust level with leveling bulb; note volume of *N*, temp., and barometric pressure, and calculate volume of *N* under standard conditions of temp. and pressure. Obtain corresponding weight of *N*, divide latter by 2, and from quotient calculate apparent percentage of amino *N* in sample. Correct result for blank test performed as above, using 10 ml of  $H_2O$  instead of the soln of the sample. The quantity of gas obtained in blank is usually 0.3–0.4 ml, and nitrite solns giving a much larger correction should be rejected.

With beef extracts and similar preparations, 5 min. is sufficient time to allow for completion of reaction in *D*. In general, the same time serves for decomposition of alpha-amino acids, but with ammonia, methylamine, and most amines other than alpha-amines 1–1.5 hours should be allowed. For determinations on such substances mix the soln of sample with reagents, as described previously, allow mixture to stand in apparatus till end of required time, and conclude reaction by shaking apparatus with the motor for 2–3 min. Continue determination as directed previously.

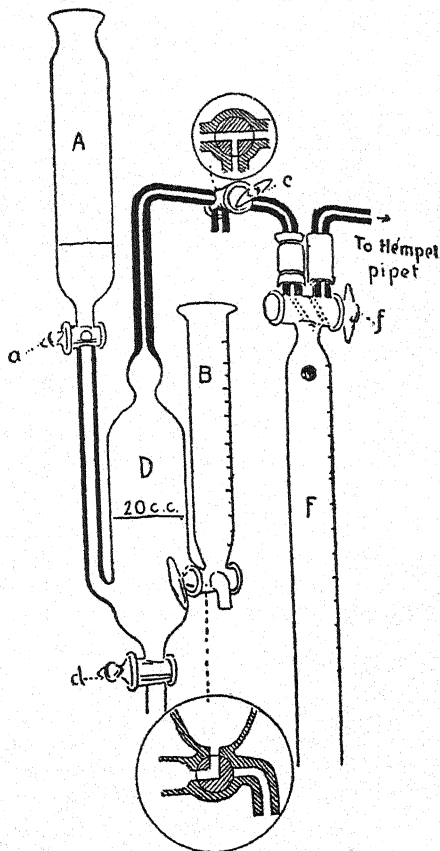


FIG. 36.—DETAILS OF DEAMINIZING BULB AND CONNECTION

To 20 ml of filtrate, 29, neutralized to phenolphthalein with  $Ba(OH)_2$  or  $NaOH$ , or to 20 ml of equivalent extract of the meat (in some cases larger volume may be

necessary) add 10 ml of freshly prepared phenolphthalein-formol mixture [50 ml of 40% formaldehyde soln containing 1 ml of a 0.5% soln of phenolphthalein in 50% alcohol, exactly neutralized with 0.2 *N* Ba(OH)<sub>2</sub> or NaOH]. Titrate mixture with 0.2 *N* Ba(OH)<sub>2</sub> soln until distinct red color appears, add slight known excess of 0.2 *N* Ba(OH)<sub>2</sub>, and titrate back to neutrality with 0.2 *N* HCl. Conduct blank titration with same reagents, using 20 ml of H<sub>2</sub>O in place of soln to be tested. From quantity of 0.2 *N* Ba(OH)<sub>2</sub> required to neutralize the mixture, corrected for quantity used in blank titration, calculate quantity of amino N present (including NH<sub>3</sub> if this has not been removed). 1 ml of 0.2 *N* Ba(OH)<sub>2</sub> soln = 2.8 mg of amino N.

36

## TOTAL SOLUBLE PHOSPHORUS—TENTATIVE

Evaporate to dryness 50 ml of the prepared water extract, 27, moisten residue with 10 ml of H<sub>2</sub>SO<sub>4</sub>, add a few drops of HNO<sub>3</sub>, and heat on hot plate until all organic matter is destroyed. Add 100 ml of H<sub>2</sub>O, boil for a few minutes, and proceed as directed under II, 9.

## 37 SEPARATION OF SOLUBLE INORGANIC AND ORGANIC PHOSPHORUS—TENTATIVE

To 500 ml of the prepared extract, 27, add 50 ml of magnesia mixture, II, 7(c), and stir thoroly. Allow to stand 15 min., add 25 ml of NH<sub>4</sub>OH, cover, and allow to stand 3 days. Filter, and wash precipitate with NH<sub>4</sub>OH (1+9). Dissolve precipitate on filter paper and that remaining in beaker in HNO<sub>3</sub> (1+1) and hot H<sub>2</sub>O, receiving soln in 400 ml beaker. Neutralize with NH<sub>4</sub>OH, make slightly acid with HNO<sub>3</sub>, add 5 g of NH<sub>4</sub>NO<sub>3</sub>, and determine inorganic P as directed under II, 9.

## MEAT EXTRACTS AND SIMILAR PRODUCTS

38

## PREPARATION OF SAMPLE—OFFICIAL

Remove liquid and semi-liquid meat extracts and similar preparations from container and mix thoroly. (A little heating expedites mixing of pasty extracts.) Carefully remove sediment that forms in many liquid preparations from bottom of container and include in sample. If sample is in form of cubes, grind 10–12 of the cubes in mortar.

39

## MOISTURE—OFFICIAL

Proceed as directed under XXVII, 2, using ca 2 g of powdered preparations, ca 3 g of pasty preparations, and 5–10 g of liquid extracts, according to solid content. Dry the powdered preparations directly without admixture. Dissolve pasty preparations in H<sub>2</sub>O and dry with sufficient ignited sand, asbestos, or pumice stone to absorb the soln. When glycerol is present, proceed as directed under XXVII, 6.

40

## ASH—OFFICIAL

Proceed as directed under XXXIV, 9 or 10. Add sufficient H<sub>2</sub>O to pasty preparations to effect soln and evaporate to dryness in order that solids may be distributed evenly over bottom of dish.

41

## TOTAL PHOSPHORUS—OFFICIAL

Destroy organic matter as directed under II, 8(c) or (d), and proceed as directed under II, 9 or 12.

42

## CHLORIDES—OFFICIAL

Dissolve ca 1 g of prepared sample, 38, in 20 ml of 5% Na<sub>2</sub>CO<sub>3</sub> soln and proceed as directed under XII, 34, 35.

43

## FAT—TENTATIVE

Transfer residue from determination of moisture to continuous extraction apparatus and proceed as directed under XXVII, 22.

44

## TOTAL NITROGEN—OFFICIAL.—See II, 21, 22 or 23.

45

## AMMONIA—TENTATIVE

Introduce 1 g of pasty extracts or 2–3 g of fluid extracts into tube B of the Folin apparatus and proceed as directed under 10.

46

INSOLUBLE NITROGEN<sup>15</sup>—TENTATIVE

Dissolve in cold H<sub>2</sub>O 5 g of powdered preparations, 8–10 g of pasty extracts, and 20–25 g of fluid extracts. Filter, and wash with cold H<sub>2</sub>O. Transfer filter paper and contents to Kjeldahl flask and determine N as directed under II, 21, 22 or 23. If a large quantity of insoluble matter is present, transfer the weighed sample to volumetric flask, dilute to definite volume, shake thoroly, filter thru folded filter, and determine N in aliquot of filtrate. Total N, 44, –N in total filtrate = N in insoluble N. Insoluble N  $\times 6.25$  = percentage of insoluble protein.

47

## COAGULABLE NITROGEN—TENTATIVE

Use as large an aliquot of the filtrate from the insoluble N, 46, as practicable, and neutralize to phenolphthalein by addition of acetic acid or NaOH, whichever may be necessary; add 1 ml of 1 N acetic acid, boil 2–3 min., cool to room temp., dilute to 500 ml, and pass thru folded filter.

Determine N in 50 ml of filtrate as directed under II, 21, 22 or 23. Soluble N (total N – N occurring as insoluble N) –  $10 \times$  the N obtained = percentage of N present as coagulable N. Coagulable N  $\times 6.25$  = coagulable protein in sample.

48

PROTEOSES AND GELATIN<sup>16</sup>—TENTATIVE

Evaporate filtrate from 47 to small volume and saturate with ZnSO<sub>4</sub> (ca 85 g to 50 ml, avoiding such an excess as would later cause bumping). Let stand several hours, filter, and wash precipitate with saturated ZnSO<sub>4</sub> soln. Place filter and precipitate in Kjeldahl flask and determine N as directed under II, 21, 22 or 23. Or, if precipitate is voluminous, which is unusual, dilute to definite volume with saturated ZnSO<sub>4</sub> soln, filter, and determine N in aliquot of filtrate as directed under II, 21, 22 or 23. N in filtrate from coagulable N, 47, –N thus obtained = N of precipitated protein (proteoses and gelatin).

49

## GELATIN—TENTATIVE

Prepare 50% soln of sample, using hot H<sub>2</sub>O, allow to cool, and place in ice box for 2 hours. If gelatin is present, the soln will set.

The ratio of total creatinin to total N in normal meat extract (1:1.5) assists in determining presence of gelatin or gelatin derivatives. The ratio is decreased when gelatin or gelatin derivatives are present in any considerable quantity.

50

## AMINO NITROGEN—TENTATIVE

Proceed as directed under 34 or 35, using aliquot of filtrate from 47.

51

ACID ALCOHOL-SOLUBLE NITROGEN<sup>17</sup>—TENTATIVE

Transfer 10 ml of an aqueous soln of sample (10 g of sample dissolved in sufficient H<sub>2</sub>O to make 100 ml), or, if sample is insoluble in H<sub>2</sub>O, 1 g of sample and 10 ml of H<sub>2</sub>O, to 200 ml glass-stoppered measuring cylinder; add 1.2 ml of 12% HCl, mix,

and add absolute alcohol to 200 ml mark. Mix thoroly and set aside for several hours. If necessary, make up to volume, filter, transfer 100 ml of filtrate to Kjeldahl flask, evaporate alcohol on water bath, and determine N in residue as directed under II, 21, 22 or 23.

52

## CREATIN—OFFICIAL

Dissolve ca 7 g of sample in cold (20°)  $\text{NH}_3$ -free  $\text{H}_2\text{O}$  in 150 ml beaker, transfer soln to 250 ml volumetric flask, dilute to mark, and mix thoroly. Transfer 20 ml aliquot of this soln to 50 ml volumetric flask and proceed as directed under 32. Subtract from combined creatinin value the equivalent of pre-formed creatinin, 53, and multiply difference by 1.16 to convert into creatin. Express result as percentage of creatin.

53

## CREATININ—OFFICIAL

Measure ca 5 ml of soln used in 52 into 500 ml volumetric flask, add 10 ml of 10%  $\text{NaOH}$  soln and 30 ml of saturated picric acid soln (1.2%), mix, and rotate for 30 seconds. Allow to stand exactly 4.5 min. and then dilute to mark at once with  $\text{H}_2\text{O}$ . Shake thoroly and read depth of color after standing. If reading is less than 7 or more than 9.5 mm, repeat, calculating quantity of soln necessary to obtain reading of ca 8 mm. Express result as percentage of creatinin, making calculations as indicated under 32.

54

NITRATES (INCLUDING ALSO NITRITES)—TENTATIVE.—See 13 or 15.

55

GLYCEROL<sup>18</sup>—TENTATIVE

Weigh 2 g of a solid or 5 g of a liquid preparation in small Pb dish or thin glass shell containing 20 g of ignited sand. Transfer dish and contents to mortar containing more ignited sand and several grams of anhydrous  $\text{Na}_2\text{SO}_4$  and mix thoroly. Transfer mixture, including dish, to Soxhlet apparatus that has a piece of cotton placed in side arm to prevent solid particles from being siphoned over. Extract entire mass with redistilled anhydrous acetone for 10 hours. Distil acetone from extract, carefully removing last trace by means of vacuum pump. Take up residue in  $\text{H}_2\text{O}$ , add 5 ml of 10%  $\text{AgNO}_3$  soln, dilute to volume of 100 ml, shake, allow to stand overnight, filter, and determine glycerol in aliquot of filtrate as directed under XXXIII, 74, beginning "Add 1 ml of  $\text{H}_2\text{SO}_4$ ." With solid meat and yeast extracts a blank of 0.5–1.0% is obtained in most cases.

56

## SUGAR—TENTATIVE

Heat 20 g of sample with ca 200 ml of  $\text{H}_2\text{O}$  on steam bath until all soluble substances have gone into soln, and proceed as directed under 23. Reducing sugars to extent of 0.5% may be present as natural constituent of meat extracts.

57

PRESERVATIVES—OFFICIAL.—See XXXII.

58

METALS—TENTATIVE.—See XXIX.

GELATIN<sup>19</sup>—TENTATIVE

59

## PREPARATION OF SAMPLE

In the case of ground gelatin mix thoroly. In the case of sheet gelatin break sheets into small pieces by hand. Further comminution is unnecessary in either case.

60

## MOISTURE

Use flat-bottomed metal dish ca 55 mm in diameter and provided with tightly

fitting slip-in cover. Heat dish and cover to constant weight at 100°, cool, and weigh. Add ca 2 g of prepared sample, 59; cover loosely, and reweigh. Place dish uncovered in water-jacketed oven and dry for 6 hours at temp. of boiling H<sub>2</sub>O. Press cover firmly in place, remove dish from oven, cool in vacuum desiccator over H<sub>2</sub>SO<sub>4</sub>, and weigh. In releasing the vacuum admit incoming air thru H<sub>2</sub>SO<sub>4</sub>. Report loss in weight as moisture.

61

## ASH

Ignite at low redness, preferably in muffle, as directed under XXXIV, 9 or 10.

62

## TOTAL PHOSPHORUS

Treat the ash, 61, with 2–3 ml of HNO<sub>3</sub> and evaporate to dryness on steam bath. Repeat the HNO<sub>3</sub> treatment and evaporation, take up residue in hot H<sub>2</sub>O containing a few drops of HNO<sub>3</sub>, and proceed as directed under II, 9.

63

## NITROGEN

Proceed as directed under II, 21, 22 or 23, using ca 2 g of sample.

64

ARSENIC<sup>20</sup>

Heat 20 g of the sample with 75 ml of As-free HCl (1+3) in covered vessel until all insoluble matter has flocculated and gelatin is dissolved. Add excess of Br water (ca 20 ml) and neutralize with NH<sub>4</sub>OH; add 0.5 ml of 85% H<sub>3</sub>PO<sub>4</sub>, or 2 g of Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, or 2 g of crystallized NaNH<sub>4</sub>HPO<sub>4</sub>·4H<sub>2</sub>O; and allow to cool. Precipitate the arsenic acid along with the phosphoric acid by adding excess (ca 30–35 ml) of magnesia mixture, II, 7(c). Allow to stand 30 min., filter, wash precipitate several times with NH<sub>4</sub>OH (1+15), drain well, and dissolve in As-free HCl (1+3) to 50 ml volume in volumetric flask. Take 25 ml aliquot, dilute to 40 ml with HCl (1+3), and proceed as directed under XXIX, 5, beginning “add 5 ml of the KI reagent.” Use HCl in preparing the standard stains and run blank determination on reagents used. Arsenic impurities, if present, are usually found in the phosphate added.

COPPER<sup>21</sup>—TENTATIVE

65

## PREPARATION OF SAMPLE

Ash 20–40 g, preferably in muffle, as directed under XXVII, 8, keeping temp low to avoid loss.

66

## REAGENT

*Standard copper soln.*—0.3927 g of recrystallized CuSO<sub>4</sub>·5H<sub>2</sub>O per liter. 1 ml = 0.1 mg of copper.

67

## DETERMINATION

Moisten the ash with small quantity of H<sub>2</sub>O, add ca 5 ml of HCl, and evaporate to dryness. Add 8 ml of HCl (1+1), heat to boiling, and transfer to 50 ml Erlenmeyer flask, using enough wash H<sub>2</sub>O to make volume ca 40 ml. Heat nearly to boiling, saturate with H<sub>2</sub>S, stopper tightly, and allow to stand in warm place for 30 min. or more. Filter into 150 ml Erlenmeyer flask and wash promptly and thoroly with warm 1:20 HCl saturated with H<sub>2</sub>S. Transfer paper and precipitate to 50 ml porcelain crucible and ignite in muffle furnace at temp. not exceeding that at which the gelatin was ashed. After ignition, cool, moisten ash with 1–2 ml of HNO<sub>3</sub>, and

evaporate to dryness on steam bath. Dissolve residue in 1 ml of  $\text{NH}_4$  acetate soln (500 g per liter). Filter into 50 ml graduated flask, wash out crucible with warm  $\text{H}_2\text{O}$ , cool, make up to mark, and mix. Measure out 25 ml into 50 ml Nessler tube, and make up to 50 ml. Add 0.2 ml of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  soln (40 g per liter) and mix. Match color against tubes prepared in same way from the standard Cu soln. Make up standards containing 1, 2, 3, 4, and 5 ml of the standard soln, equivalent to 10, 20, 30, 40, and 50 p.p.m. of Cu if a 20 g sample is used and one-half of soln taken. Solutions giving a stronger reaction than 6 ml of the standard cannot be accurately compared. If a reaction stronger than that given by 6 ml of the standard is obtained, take aliquot smaller than 25 ml and repeat determination.

## 68

ZINC<sup>21</sup>—TENTATIVE

Boil filtrate and washings from the  $\text{H}_2\text{S}$  precipitate of Cu until all  $\text{H}_2\text{S}$  is removed. Add 1 ml of  $\text{HNO}_3$  and continue boiling until volume is reduced to ca 25 ml. Add 10 ml  $\text{NH}_4\text{Cl}$  (200 g per liter), make definitely alkaline with  $\text{NH}_4\text{OH}$ , heat nearly to boiling, and filter into 100 ml Erlenmeyer flask. Wash with warm alkaline  $\text{NH}_4\text{Cl}$  soln containing 50 g of  $\text{NH}_4\text{Cl}$  and 25 ml of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90) per liter. Neutralize filtrate and washings with acetic acid, and add 0.5 g of Na acetate and sufficient glacial acetic acid to make excess of 2 ml for each 50 ml of soln. Warm mixture on steam bath and saturate with  $\text{H}_2\text{S}$ . Allow to stand in warm place ca 30 min. Filter thru small paper and wash thoroly with warm dilute acetic acid saturated with  $\text{H}_2\text{S}$ . If filtrate is turbid, return to flask, add a few drops of saturated  $\text{HgCl}_2$  soln, shake, and filter again. Ignite in tared Pt crucible at a dull red heat until completely ashed, then a few minutes at  $950$ – $1000^\circ$ . Weigh as  $\text{ZnO}$ . Weight of  $\text{ZnO} \times 40,000 =$  p.p.m. of Zn if 20 g sample was taken.

## 69

POLARISCOPIC CONSTANTS<sup>22</sup>

Prepare soln of concentration of 3 g per 100 ml by soaking 3 g of sample in 40–50 ml of cold  $\text{H}_2\text{O}$  ca 15 min., heating to complete soln at ca  $50^\circ$  and diluting to volume of 100 ml at  $35^\circ$ . Polarize at  $35^\circ$  in 2 dm tube, using Ventzke scale.

Cool portion of gelatin soln rapidly to  $10$ – $15^\circ$  and pour into cold, dry 1 dm tube before jelly has had time to form. Place tube in constant temp. bath at  $15^\circ$  for 18 hours to obtain equilibrium rotation, and then polarize at  $15^\circ$ . Double the reading to place it on basis of a 2 dm tube.

In order to clarify cloudy samples before polarizing, digest original 100 ml in stoppered flask with ca 10 g of lightly powdered  $\text{MgCO}_3$  for at least 1 hour at  $35$ – $40^\circ$  and filter thru folded filter until clear, avoiding unnecessary evaporation. The increase in laevorotation (mutarotation) between  $35^\circ$  and  $15^\circ$  is an index of jelly strength developed.

## SULFUR DIOXIDE

## 70

*Distillation Method.*—See XXXII, 32.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 13, 42 (1930).
- <sup>2</sup> Ibid., 16, 75, 543 (1933); 17, 70 (1934); 18, 82 (1935).
- <sup>3</sup> Ibid., 11, 408 (1928).
- <sup>4</sup> Ibid., 1, 174 (1915).
- <sup>5</sup> Tiemann, Anleitung zur Untersuchung von Wasser, 1870, p. 56; Wiley, Principles and Practice of Agricultural Analysis, 2nd ed., 1908, vol. 2, p. 397; U. S. Dept. Agr. Bur. Chem. Bull. 13 (X), p. 1403; J. Assoc. Official Agr. Chem., 4, 502 (1921); 6, 74 (1922).
- <sup>6</sup> J. Assoc. Official Agr. Chem., 22, 82 (1939).

- <sup>7</sup> Ibid., 8, 277, 696 (1925).
- <sup>8</sup> U. S. Dept. Agr. Bur. Chem. Bull. 162, p. 97.
- <sup>9</sup> Abs. Z. Nahr. Untersuch. Hyg. Warenkunde, 10, 173 (1896).
- <sup>10</sup> J. Ind. Eng. Chem., 2, 21, 215 (1910).
- <sup>11</sup> C. A., 4, 218 (1910).
- <sup>12</sup> J. Am. Chem. Soc., 28, 1485 (1906).
- <sup>13</sup> J. Biol. Chem., 9, 185 (1911); 12, 275 (1912); 16, 121 (1913); 23, 407 (1915).
- <sup>14</sup> Biochem. Z., 7, 45 (1907).
- <sup>15</sup> Allen, Commercial Organic Analysis, 4th ed., 1909-14, vol. 8, p. 407.
- <sup>16</sup> Z. Anal. Chem., 34, 562 (1895); U. S. Dept. Agr. Bur. Chem. Bull., 54.
- <sup>17</sup> J. Am. Chem. Soc., 36, 1551 (1914).
- <sup>18</sup> J. Assoc. Official Agr. Chem., 1, 279 (1915).
- <sup>19</sup> Ibid., 5, 343 (1922); J. Ind. Eng. Chem., 15, 942 (1923).
- <sup>20</sup> U. S. Dept. Agr. Bur. Chem. Circ., 102; J. Soc. Chem. Ind., 26, 1115 (1907).
- <sup>21</sup> Ind. Eng. Chem., 15, 942 (1923); J. Assoc. Official Agr. Chem., 22, 84 (1939).
- <sup>22</sup> J. Assoc. Official Agr. Chem., 4, 520 (1921).



## XXIX. METALS IN FOODS

### ARSENIC

#### *Gutzeit Method—Official*

1

#### REAGENTS

(a) *Stannous chloride soln.*—Dissolve 40 g of As-free  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  in HCl and make up to 100 ml with the same strength acid.

(b) *Zinc.*—Use 20- or 30-mesh, As-free granulated Zn, that needs no preliminary treatment, or As-free stick Zn either cut into equal 1 cm lengths, or melted and cast into pellets in porcelain mold drilled (for example) 9 mm in diameter and 12.5 mm deep. Activate the pieces of Zn with HCl (1+3), to which has been added 2 ml of the  $\text{SnCl}_2$ , allowing action to continue 15 min. Sort out distinctly inactive or over-active pieces and pour off liquid. Wash Zn free from acid with clear tap  $\text{H}_2\text{O}$ , and rinse with hot  $\text{H}_2\text{O}$ . Select uniformly etched non-pitted Zn and store in suitable receptacle. To maintain supply of uniform Zn adopt a system of rotation by withdrawing Zn from the original receptacle until stock is exhausted and storing used Zn in a second receptacle after discarding non-uniform or deeply pitted pieces. Draw Zn from second receptacle after washing it with clear running  $\text{H}_2\text{O}$ . Repeat procedure until pieces are too small for further use.

(c) *Ammonium oxalate soln.*—Saturated.

(d) *Potassium iodide soln.*—Dissolve 15 g of KI in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(e) *Sand.*—Clean 30-mesh (thru 30- but not 40-mesh) white sea sand by washing successively with hot 10% NaOH soln, hot concentrated  $\text{HNO}_3$ , and hot distilled  $\text{H}_2\text{O}$ . Dry the clean sand.

(f) *Mercuric bromide paper.*—Use commercial arsenic papers cut from paper of uniform weight and texture into strips exactly 2.5 mm wide and ca 12 cm long. (Uniformity in width and texture of paper are of great importance in this comparison method. Irregular texture produces irregular impregnation with consequent inaccurate results.) To sensitize, soak strips 1 hour or longer in 3-6% (optimum 5%) soln of filtered  $\text{HgBr}_2$  in alcohol, according to quantity, character, and activity of Zn used. (Attenuated, unsatisfactory stains, due to over rapid evolution of arsine, can be shortened and intensified by increasing concentration of  $\text{HgBr}_2$  and vice versa.) If the strips are in sheets, cut off two sides before soaking and leave strips attached at ends. After sensitization remove strips and dry individual ones on glass rods, and groups by waving them in the air. Place strips when nearly dry between clean sheets of paper and subject them to pressure long enough to take out bends or curls. Store in dry dark place. (Aging of impregnated strips usually results in markedly fainter and longer stains. Desirable types of stain result from use of impregnated strips not over 2 days old.) When ready for use, cut individual strips off squarely half an inch from one end and insert this end into the narrow tube of apparatus. Handle sheets by the paper attached to either end and cut in half just before use. Strips must be clean and free of any contamination.

(g) *Standard arsenic soln.*—Dissolve 1 g of  $\text{As}_2\text{O}_3$  in 25 ml of 20% NaOH. Saturate soln with  $\text{CO}_2$  and dilute to 1 liter with recently boiled  $\text{H}_2\text{O}$ . 1 ml of this soln contains 1 mg of  $\text{As}_2\text{O}_3$ . Dilute 40 ml of this soln to 1 liter. Make 50 ml of the diluted soln to 1 liter and use to prepare standard stains. 1 ml of latter soln contains 0.002 mg of  $\text{As}_2\text{O}_3$ . A soln containing 0.001 mg of  $\text{As}_2\text{O}_3$  may also be prepared if desired. Prepare fresh dilute solns at frequent intervals.

## 2

## APPARATUS

(a) *Generators and absorption tubes.*—Use 2 oz wide-mouthed bottles of uniform capacity and design as generators, and fit each by means of perforated stopper with glass tube 1 cm in diameter and 6–7 cm long, with an additional constricted end to facilitate connection. Place small wad of glass wool in constricted bottom end of tube and add 3.5–4 g of the 30-mesh cleaned sand, taking care to have same quantity in each tube. Moisten sand with 10% Pb acetate soln and remove excess by light suction. Clean sand when necessary by treatment (do not remove sand from tube) with  $\text{HNO}_3$  followed by  $\text{H}_2\text{O}$  rinse and suction. Treat with the Pb acetate soln. If sand has dried thru disuse, clean and remoisten it as directed. Connect tube by means of rubber stopper with narrow glass tube 2.6–2.7 mm in internal diameter and 10–12 cm long, and introduce the clean end of the strip of  $\text{HgBr}_2$  paper. (A 3 mm bore allows strip to curl, which results in an uneven stain and poor end point.) Clean and dry tube before inserting the bromide paper. (An ordinary pipe cleaner may be used.)

(b) *Water bath.*—Use any constant temp. water bath. If no water bath is available, use any flat-bottomed container of suitable depth and capacity. (A deep water bath is suggested to insure uniform conditions during evolution and absorption of the As.)

## 3

## PREPARATION OF SAMPLE

(a) *For fresh fruits (apples, pears or similar products).*—Weigh and peel representative sample of fruit (1–5 lbs.). At blossom and stem ends cut out all flesh thought to be contaminated with arsenical compounds and include with peelings. Place peelings in 1 or more 800 ml Pyrex Kjeldahl flasks. (As-free Pyrex glassware and “wet ashing” apparatus of Duriron are now available.) Add 25–50 ml of  $\text{HNO}_3$ , then add cautiously 20 ml of  $\text{H}_2\text{SO}_4$ . Place each flask on an asbestos mat with 2" hole. Warm slightly and discontinue heating if foaming becomes excessive. When reaction has quieted, heat cautiously and rotate flask from time to time to prevent caking of sample upon glass exposed to flame. Maintain oxidizing conditions in flask at all times during digestion by adding cautiously small quantities of  $\text{HNO}_3$  whenever mixture turns brown or darkens. Continue digestion until organic matter is destroyed and  $\text{SO}_3$  fumes are copiously evolved. (Final soln should be water-white, or at most a light straw color.) Cool slightly and add 75 ml of  $\text{H}_2\text{O}$  and 25 ml of the saturated soln of  $\text{NH}_4$  oxalate to assist in expelling oxides of N from the soln. Evaporate again to point where fumes of  $\text{SO}_3$  appear in neck of flask. Cool, and dilute with  $\text{H}_2\text{O}$  to 500 or 1000 ml in volumetric flask.

(b) *For dried fruit products.*—Prepare sample by alternately grinding and mixing 4–5 times in food chopper. Place 35–70 g portions in 800 ml Kjeldahl flasks, and add 10–25 ml of  $\text{H}_2\text{O}$ , 25–50 ml of  $\text{HNO}_3$ , and 20 ml of  $\text{H}_2\text{SO}_4$ . Continue digestion as directed in 3(a). Dilute digested soln to 250 ml.

(c) *For small fruits, vegetables, etc.*—Use 70–140 g of sample and digest as directed under (a) and (b).

(d) *For materials other than (a), (b), or (c).*—Digest 5–50 g, according to degree

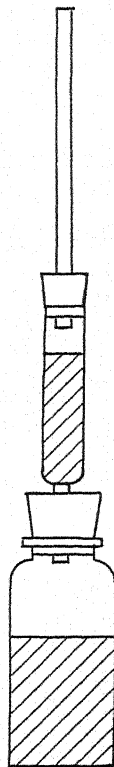


FIG. 37.—GENERATOR TO BE USED WITH GUT-ZEIT METHOD FOR DETERMINATION OF ARSENIC.

of dryness and amount of As expected, as directed under 3(a) and (b). Dilute to definite volume dictated by circumstances.

(e) *For products containing stable organic As compounds, products liable to yield incompletely oxidized organic derivatives that inhibit arsine evolution, or products that are otherwise especially difficult to digest.*—Shrimp, tobacco, oils, and sometimes other products require special treatment to complete oxidation of organic As to inorganic  $\text{As}_2\text{O}_3$ , or to destroy organic interferences previous to As determination. For details consult following references:

(1) C. R. Gross, *Ind. Eng. Chem. Anal. Ed.*, 5, 58 (1933).

(2) Carey, Blodgett, and Satterlee, *Ibid.*, 6, 327 (1934).

(3) Remington, Coulson, and von Kolnitz, *Ibid.*, 280.

(4) C. C. Cassil, *J. Assoc. Official Agr. Chem.*, 20, 171 (1937).

Dilute the As solns obtained by these special methods of preparation to definite volume.

#### 4

#### ISOLATION OF ARSENIC

Isolate the As when interfering substances are present in digests (pyridine from tobacco), or when samples contain excessive amounts of salts, or  $\text{H}_2\text{SO}_4$  from digestions, before making determinations. Consult reference 3(1), or use trichloride distillation of bromate method, 6.

#### 5

#### DETERMINATION

Determine the acid ( $\text{HCl}$  or  $\text{H}_2\text{SO}_4$  according to previous treatment), by titration if necessary, in definite volume of sample soln. Place aliquots containing 0.01–0.03 mg of  $\text{As}_2\text{O}_3$  (0.020–0.025 mg is optimum) and not larger than 30 ml in Gutzeit generators. If arsenic in aliquot taken is found to be outside the limits specified, repeat with proper aliquot. If aliquot contains only  $\text{HCl}$ , add sufficient  $\text{HCl}$  to make total volume of 5 ml; if it contains  $\text{H}_2\text{SO}_4$ , add sufficient 25% As-free  $\text{NaOH}$  soln (keep in As-free Pyrex) to exactly neutralize it and add 5 ml of  $\text{HCl}$ , or add sufficient  $\text{HCl}$  to the  $\text{H}_2\text{SO}_4$  in aliquot to make total volume of 5 ml. Cool when necessary and add 5 ml of the  $\text{KI}$  reagent and 4 drops of the  $\text{SnCl}_2$ , 1(a). Prepare standards corresponding to 0.010, 0.020, and 0.030 mg of  $\text{As}_2\text{O}_3$  from Reagent (g). Since the standards *must* contain same kind and amounts of acid as samples, add 5 ml of  $\text{HCl}$ , or  $\text{H}_2\text{SO}_4$  and  $\text{HCl}$  (total 5 ml) according to prior treatment of unknown. If the  $\text{H}_2\text{SO}_4$  has been neutralized, add an equivalent quantity of As-free  $\text{Na}_2\text{SO}_4$  to standards. Mix, and allow to stand for 30 min. at not less than  $25^\circ$  or 5 min. at  $90^\circ$ . Dilute with  $\text{H}_2\text{O}$  to 40 ml.

Prepare generator as directed under 2 and center strip of  $\text{HgBr}_2$  paper carefully in the narrow tube. According to activity of the Zn, add to each of standards and samples 10–15 g of activated stick Zn or 2–5 g of granulated Zn and add the same quantity to each generator. Equalize as far as possible surface area of Zn exposed in standard and sample. If sheets of strips are used, prepare sample and standard strips from same strip-group.

Immerse apparatus to within 1" of top of narrow tube in water bath, which is kept at constant temp. of  $20$ – $25^\circ$ , and allow evolution to proceed for 1.5 hours. Remove strip and average length of stains on both sides in mm. Plot graph of standard strips on cross-sectioned paper, using length in mm as ordinate and the mg of  $\text{As}_2\text{O}_3$  as abscissa. (Preparation of standard graph averages errors of individual standards. Reading strip from such a graph is considered more convenient and accurate than comparing strips themselves.) Locate length of unknown strip on standard graph and read off on abscissa quantity of As present. Report only to third decimal as grains of  $\text{As}_2\text{O}_3$  per pound. Take smaller or larger aliquots when

stain is longer or shorter than highest or lowest standard, respectively. Grain/lb.  $\times 143 = \text{p.p.m.}$ ;  $\text{p.p.m.} \times 0.007 = \text{grain/lb.}$

Frequent blanks should be made. With reagents of suitable quality, blanks should not show more than 0.001 mg of  $\text{As}_2\text{O}_3$ .

### Bromate Method<sup>2</sup>—Tentative

(Applicable to determination of arsenic in plants and food products where a sample of convenient size for digestion will yield at least 0.005 grain (0.324 mg) of  $\text{As}_2\text{O}_3$ .)

#### 6

##### REAGENTS

(a) *Ammonium oxalate-urea soln.*—To saturated  $\text{H}_2\text{O}$  soln of  $\text{NH}_4$  oxalate add 50 g of urea per liter.

(b) *Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g of hydrazine sulfate and 20 g of  $\text{NaBr}$  in 1 liter of  $\text{HCl}$  (1+4).

(c) *Sodium chloride.*—Commercial salt, uniodized.

(d) *Standard potassium bromate soln.*—Dissolve 0.1823 g of  $\text{KBrO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. 1 ml = 0.005 grain of  $\text{As}_2\text{O}_3$ . Standardize by titration against the standard  $\text{As}_2\text{O}_3$  soln, (e), making titration at ca  $90^\circ$  and in presence of ca 100 ml of  $\text{H}_2\text{O}$  and 25 ml of  $\text{HCl}$ , in order to simulate conditions under which samples will be titrated. 1 ml of the bromate soln should be equivalent to 1 ml of  $\text{As}_2\text{O}_3$  soln.

(e) *Standard arsenious oxide soln.*—Dissolve 0.3241 g of  $\text{As}_2\text{O}_3$  in 25 ml of 10%  $\text{NaOH}$ , make slightly acid with  $\text{H}_2\text{SO}_4$  (1+6), and dilute with  $\text{H}_2\text{O}$  to 1 liter.

#### 7

##### DISTILLING APPARATUS

The distilling apparatus consists of 800 ml Kjeldahl flask (A), distilling tube (B), and 300 ml Erlenmeyer flask (C).

To prepare distilling tube, bend 10–15 mm glass tube to acute angle of ca  $70^\circ$ . Draw the longer arm, which is ca 15–20" long, down to orifice of ca 3 mm. Fit shorter arm (ca 4") with No. 7 rubber stopper, which has previously been boiled in 10%  $\text{NaOH}$  for 15 min., and then in  $\text{HCl}$  for 15 mm., in order to remove most of the sulfur compounds which might be distilled and react with the bromate soln.

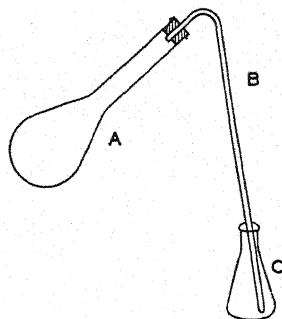


FIG. 38.—DISTILLING APPARATUS FOR DETERMINATION OF ARSENIC BY BROMATE METHOD

#### 8

##### PREPARATION OF SAMPLE

Introduce a suitable sample containing 0.005 grain (0.324 mg) or more of  $\text{As}_2\text{O}_3$  into an 800 ml Kjeldahl flask. Proceed with acid digestion as directed under 3, with following exception: Add exactly 20 ml of  $\text{H}_2\text{SO}_4$ , or (rarely), if material is difficult to digest, exactly 25 ml at beginning of digestion. After digestion is complete, add 50 ml of  $\text{H}_2\text{O}$  and 25 ml of the  $\text{NH}_4$  oxalate-urea soln, and boil until white  $\text{SO}_3$  fumes extend up into neck of flask to decompose oxalates and urea completely. (Volatile intermediate products may titrate with bromate. If heat available is insufficient to decompose these substances, it is preferable to evaporate to fumes with  $\text{H}_2\text{O}$  alone. Hydrazine sulfate will destroy small amounts of oxides of nitrogen.)

## 9

## ISOLATION

Add 25 ml of  $H_2O$  to digested soln in the Kjeldahl flask and cool to room temp. Put 100 ml of  $H_2O$  into flask C. Add to soln in Kjeldahl flask 20 g of  $NaCl$  and 25 ml of the hydrazine sulfate-sodium bromide soln and connect distilling tube. Heat Kjeldahl flask over small well-protected flame, and distil into the  $H_2O$  in Erlenmeyer flask. (Heating is not intended to boil soln but to bring about evolution of  $HCl$  gas, which carries over the  $AsCl_3$  with it. Absorption of evolved  $HCl$  gas by  $H_2O$  causes rise in temp., which indicates progress of distillation.) Adjust flames so that temp. of distillate soln will rise to  $90^\circ$  in 9–11 min. and then discontinue distillation. (Residual mixture in flask should not be less than 55 ml.) If distillation proceeds further, or larger quantity of  $H_2SO_4$  than that specified is used in the digestion,  $SO_2$  is distilled, which is titrated as As.

## 10

## DETERMINATION

Titrate distillate at once with the bromate soln, using 3 drops of methyl orange indicator. (Single drops of indicator, VI, 3(f), but not exceeding 3, may be added during titration as the red color fades.) Towards end of titration add the bromate soln very slowly and with constant agitation to prevent local excess. The end point is reached when a single drop of the bromate just destroys the final tinge of red color. Use Erlenmeyer flask containing clear  $H_2O$  for comparison. (End point must not be exceeded as action of indicator is not reversible and back titrations are not reliable. At the proper end point, the red color produced by 2 additional drops of methyl orange indicator should persist for at least 1 min.) Correct results for volume of bromate used in blank run (digest 5 g of pure sucrose) with the same reagents (same quantities) and regular distillation procedure. (The blank titration should not exceed 0.7 ml of bromate soln. The method is accurate down to variations in blank, which should not exceed 0.1 ml when chemicals from same lot are used.) Should blank titration be high or variable, test individual reagents for purity by bromate titration and discard unsatisfactory ones. Test the  $H_2SO_4$  by bringing 20 ml to boil, cooling, diluting with  $H_2O$  to 100 ml, adding a little  $HCl$ , and titrating while hot. It probably will furnish most of the blank. Select rubber stoppers carefully as they are often the source of unsatisfactory blanks.

If high results, due to  $SO_2$  produced during distillation, or other reducing substances, are suspected, dilute titrated distillate to definite volume and redetermine the As in aliquot by Gutzeit method, 1–5. A positive test for sulfates in an aliquot of the titrated distillate indicates contamination with reduced S compounds and a necessity for check on the As. All glass apparatus will reduce blanks to minimum.

LEAD<sup>2</sup>—TENTATIVE

## 11

## PRINCIPLES

The general method calls for ashing, 14, separation of the Pb, either as the dithizone complex, 16, or as the sulfide, 17, followed (depending upon quantity) by electrolytic determination, 18, 19, 20, or by colorimetric dithizone determination in comparator tubes, 22, or with a photometer, 23. The subject of interference is treated separately, 24–26, and the analyst should familiarize himself with the details of these sections before applying the method. Special methods of preparation are presented under 27–30.

## 12

## PRECAUTIONS

The analyst should decide whether nature of determination requires unusual precautions in purification of reagents, or whether blank determination will be suffi-

cient. The smaller the quantity of Pb to be determined, the greater the care required in reduction of blank (see also 21).

To test suitability of any reagent place 15–20 ml of concentrated acids or 10–15 g of solid reagents dissolved in redistilled  $H_2O$  in separatory funnel and add sufficient Pb-free citric acid to prevent precipitation by ammonia of iron, aluminum, alkaline earth phosphates, or other substances. Make soln ammoniacal and add 2–3 ml of 10% KCN. Shake soln with ca 5 ml of dithizone soln, 13(e) (5–10 mg per liter). If lower layer is green, transfer it to another separatory funnel and extract excess dithizone with  $NH_4OH$  (1+99) to which has been added a drop of KCN soln. If  $CHCl_3$  layer is colorless, consider test negative for general analytical purposes.

When special purification becomes necessary, redistil  $H_2O$  (distilled  $H_2O$  stored in tin-lined tanks usually contains Pb and Sn),  $NH_4OH$ ,  $HNO_3$ ,  $HCl$ ,  $HBr$ ,  $Br$ , and  $CHCl_3$  (U.S.P. free from chlorides) in resistant, all-glass stills (Pyrex is suitable). If stills are new, steam them out with hot  $HCl$  or  $HNO_3$  vapors to remove "surface" Pb. (Subsequent distillates may not be totally Pb-free.) Purify citric acid, Na or  $NH_4$  acetate,  $Al(NO_3)_3$ ,  $Ca(NO_3)_2$ , and  $Na_2SO_4$  by precipitating the Pb from their aqueous solns with  $H_2S$ , using 5–10 mg of  $CuSO_4$  as a coprecipitant (citric acid and aluminum nitrate solns require adjustment with ammonia to pH 3.0–3.5, bromophenol blue indicator). Filter (fritted glass filter is most convenient), boil filtrates for 20 min. to expel excess  $H_2S$  and filter again if necessary to obtain brilliantly clear solns. Purify other reagents by recrystallization.

Store redistilled acids or purified solns of reagents in resistant glass containers of minimum Pb content (Pyrex is suitable) carefully cleaned of surface Pb with hot  $HNO_3$ .

Clean new glass and chemical ware carefully with hot 10%  $NaOH$  soln followed by hot  $HNO_3$  and use only for Pb determinations.

In preparation of samples for analysis, avoid Pb contamination. If mixing or grinding is necessary, use porcelain mortar if possible. Avoid use of metal food grinders unless previous experiment has shown that no contamination of sample with Pb or Sn results. If product to be analyzed cannot be thoroly mixed in its own container, or if composite sample of a number of containers is desired, empty into large glass jar or porcelain dish and mix thoroly with wooden spoon or porcelain spatula. If liquid portion of sample cannot be incorporated into ground solid material to obtain homogeneous mixture, analyze separately. If the food is packed in tins having soldered seams (sardines and meats), open tins from bottom to avoid contaminating sample with bits of solder. Avoid sifting in preparation of samples to prevent metallic contamination or segregation of Pb.

## GENERAL METHOD

### *Sn and Bi Absent*

(Applicable to such materials as carbohydrates, cereals and cereal products, cacao and dairy products, feeds, meats, fish, plant material, fruit and fruit products, fresh vegetables, etc., and in general to all organic materials (except fats) in which no Sn and Bi are encountered. For products containing Sn (canned foods) or Bi, proceed as directed under 24–26.)

## 13

### REAGENTS

(a) *Standard lead solns.*—Dissolve 20–50 g of C.P.  $Pb(NO_3)_2$  in minimum of hot  $H_2O$  and cool with stirring. Filter crystals with suction on small Büchner funnel, redissolve, and repeat the recrystallization. Dry crystals at 100–110° to constant weight. Cool in desiccator and preserve in tightly stoppered bottle. (The product has no water of crystallization and is not appreciably hygroscopic.) Prepare stock soln containing equivalent of 2 mg of Pb [3.197 mg of  $Pb(NO_3)_2$ ] per ml in 1%

HNO<sub>3</sub> (b). Prepare weaker dilutions with 1% HNO<sub>3</sub> as needed, and do not store over long periods, because Pb tends to precipitate out.

(b) *Nitric acid*.—1%. Dilute 10 ml of fresh, water-white HNO<sub>3</sub> (sp. gr. 1.40) to 1 liter with redistilled H<sub>2</sub>O. If acid has been redistilled, boil off nitrous fumes and readjust to 1.40 sp. gr. by evaporation or dilution.

(c) "*Ash Aid*" soln.—Dissolve 40 g of Al(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O + 20 g of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O in 100 ml of H<sub>2</sub>O.

(d) *Citric acid*.—Concentrated Pb-free soln. 1 ml = 0.5 g of citric acid (reagent partially neutralized with NH<sub>4</sub>OH during purification, 12).

(e) *Diphenylthiocarbazone (dithizone)*.—Dissolve ca 1 g of the commercial reagent in 50–75 ml of CHCl<sub>3</sub> and filter if insoluble material remains. Shake out in separatory funnel with four 100 ml portions of metal-free (redistilled) NH<sub>4</sub>OH (1+99). Dithizone passes into aqueous phase to give orange colored soln. Filter aqueous extracts into large separatory funnel thru pledget of cotton inserted in stem of a funnel. Acidify slightly with dilute HCl and extract precipitated dithizone with two or three 20 ml portions of CHCl<sub>3</sub>. Combine the extracts in a separatory funnel and wash two or three times with H<sub>2</sub>O. Draw off into beaker and evaporate the CHCl<sub>3</sub> with gentle heat on steam bath, avoiding spattering as soln goes to dryness. Remove last traces of moisture by heating for an hour at not over 50° in vacuo. Store the dry reagent in dark in tightly stoppered bottle. Make up the reagent solns for extraction to contain 100, 50, and 10 mg per liter in freshly redistilled CHCl<sub>3</sub> and store in dark at 5–10°. (A stock soln of dithizone in CHCl<sub>3</sub> containing 1 mg per ml will keep a long time and is convenient for use in making dilutions.)

(f) "*Stripping*" reagent.—To 20 ml of saturated Na acetate soln, add 10 ml of glacial acetic acid and make to 100 ml.

(g) *Potassium iodide soln*.—2%. Prepare as frequently as is necessary to prevent formation of a starch-iodine color when mixed with reagent (f) in proportions specified in 20.

(h) *Starch soln*.—Make up 1 g of soluble starch to 200 ml.

(i) *Sodium thiosulfate*.—Approximately 0.1 N stock soln. Dissolve 24.8 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in 1 liter of CO<sub>2</sub>-free H<sub>2</sub>O and allow it to stand (preferably for 2 weeks) before use. Prepare ca 0.001 and 0.005 N solns by dilution of the stock soln in exact ratios of 1:100 and 1:20 with CO<sub>2</sub>-free H<sub>2</sub>O and standardize these electrolytically, using standard Pb soln equivalent to 0.2–1.0 mg of Pb for the 0.001 N dilution and 1–5 mg of Pb for the 0.005 N dilution. Subtract anode blanks, 19–20, and take as the thiosulfate factor the average number of mg of Pb equal to 1 ml of the solns. Make fresh dilutions daily and check the Pb factor at least every month.

(j) *Ammonia-cyanide mixture*.—To 100 ml of 10% recrystallized, phosphate-free KCN or NaCN in 500 ml volumetric flask add sufficient redistilled NH<sub>4</sub>OH to introduce 19.1 g of NH<sub>3</sub> and complete to volume with redistilled H<sub>2</sub>O. (Strength of redistilled NH<sub>4</sub>OH can be determined by sp. gr. or titration.)

(k) *Pure metallic tin*.—Purest obtainable, such as Bureau of Standards Sample No. 42 B (0.0035% Pb). Granulate tin as finely as possible by melting and pouring very slowly into H<sub>2</sub>O. Determine Pb content as follows: Dissolve 1–2 g sample in HBr or HCl and volatilize the Sn by evaporating soln to dryness and treating with several 5 ml portions of the HBr-Br<sub>2</sub> mixture, (l), evaporating to dryness on steam bath after each treatment. Take up with 2–3 ml of HNO<sub>3</sub>, evaporate to dryness to expel Br, and take up with hot H<sub>2</sub>O. Filter, adjust acidity to 1% with HNO<sub>3</sub> and proceed as directed in 19 and 20.

(l) *Hydrobromic acid-bromine mixture*.—To 250 ml of 40% redistilled HBr add 35 ml of redistilled liquid Br.

(m) *Sodium polysulfide*.—Dissolve 480 g of Na<sub>2</sub>S·9H<sub>2</sub>O and 40 g of NaOH in

H<sub>2</sub>O, add 16 g of powdered S, shake until S dissolves, filter, and dilute to 1 liter.

(n) *Hydrochloric acid-citric acid*.—Add a quantity of Reagent (d) equivalent to 50 g of citric acid to 50 ml of HCl and dilute to 250 ml.

(o) *Sodium oleate soln.*—10%. To 45 ml of 30% NaOH and 400 ml of H<sub>2</sub>O in 1.5 liter beaker, add slowly while heating and stirring 90 g (by difference from a separatory funnel) of oleic acid. Heat mixture on steam bath until soap is entirely dissolved. (A small flocculent precipitate of impurities may remain.) Cool, dilute to 1 liter, mix, and filter.

(p) *Ammonia-cyanide-citrate soln.*—Dissolve 10 g of recrystallized phosphate-free KCN or NaCN and 10 g of citric acid in 500 ml of NH<sub>4</sub>OH (sp. gr. 0.90) and dilute to 1 liter. Preserve in dispensing apparatus that will minimize loss of NH<sub>3</sub> by volatilization.

## 14

## PREPARATION OF SAMPLE (ASHING)

The quantity of material taken for a sample depends upon amount available and expected Pb content, and whether the Pb is to be determined as directed in 19 and 20 or 22 and 23. In general, weigh a representative sample of 5–200 g, depending upon conditions, into porcelain dish or casserole of convenient size. Dry wet samples on steam bath or in hot water oven. Add 2–5 ml of the “ash-aid” soln, 13(c), to products difficult to ash (meats), or to furnish ash bulk to low ash products (candies and jellies low in fruit content); mix well, and dry. Char gelatin, carbohydrate foods such as jam, and other products that have a tendency to swell excessively, by carefully heating over a burner. (Swelling can be controlled by playing a small flame from a glass jet over surface of the material in the dish, but a metallic burner must not be used for this purpose because of possible metallic contamination.) Do not allow material to ignite. Milk, candies, etc., may be charred without ignition by adding the sample a little at a time to casserole heated over burner or hot plate. When samples are dry or charred, place them in temp.-controlled muffle and raise temp. *slowly* to 500° without ignition. If sample contains fat, “smoke” it away by heating sufficient length of time at ca 350°. Cover floor of muffle with piece of asbestos board or silica plate so that sample receives most of its heat by radiation from sides and roof and not by conduction from hotter floor of muffle.

If muffle is provided with automatic control, conduct ashing overnight at not over 500°. If sample is not completely ashed the next morning or if day-time ashings at 500° are not proceeding satisfactorily, remove casserole, cool, and moisten char with 2–5 ml of the ash-aid. Dry contents of casserole past danger of spattering (no free liquid) and replace it in muffle. If ashing is not complete or proceeding rapidly after 30 min., remove casserole, cool, and cautiously add 2–3 ml of HNO<sub>3</sub>. Dry, place in muffle, and continue ashing until practically carbon-free. Avoid excessive use of ash-aid and particularly HNO<sub>3</sub>, if sample still contains much intermixed carbon, because local overheating or deflagration may result, especially if much potassium is present in ash.

When a clean ash is obtained, cool, cover casserole with watch-glass, and add cautiously 15–20 ml of HCl. Rinse down watch-glass with H<sub>2</sub>O and heat on steam bath. If a *clear* soln is not obtained, evaporate again to dryness and repeat addition of HCl. If insoluble matter persists, evaporate HCl and dehydrate silica by heating to fumes with 5–10 ml of 60% HClO<sub>4</sub> (double distilled preferred). If HClO<sub>4</sub> is used, considerable H<sub>2</sub>O (200 ml) may be necessary to completely dissolve KClO<sub>4</sub> later, especially if KCN instead of NaCN is used in the dithizone extraction of Pb, 16.

Dilute with H<sub>2</sub>O and filter soln when necessary with suction thru fritted glass filter (Jena 11G4 is preferable). Catch filtrate in 500 ml glass-stoppered Erlenmeyer



flask under a bell-jar. Leach insoluble material on filter successively with a few ml of hot HCl, the hot HCl-citric acid soln, and hot 40%  $\text{NH}_4$  acetate.

In certain instances take following special precautions:

(1) If quantity of insoluble material (silica) remaining on filter is abnormal, flush it into Pt dish with  $\text{H}_2\text{O}$ , evaporate, and treat residue with one or two 5 ml portions of HF. Evaporate to dryness and take up residue with  $\text{H}_2\text{O}$  and a few ml of HCl or  $\text{HClO}_4$  and add to bulk of ash filtrate. (2) When ashing is of long duration, no ash-aid has been used, or natural ash is low with little ash bulk, Pb may be baked on dish. To remove this Pb, add a few pellets (2–3 g) of NaOH and dissolve in a few ml of hot  $\text{H}_2\text{O}$ . Tilt dish so that sirupy soln completely wets that portion of interior originally occupied by sample, then heat for short time on steam bath, but do not bring to dryness. (Overheating with strong NaOH may result in extracting a few micrograms of Pb from casserole. Porcelain retains Pb to a less extent than does silica but may contain very small quantities of Pb.) Take up residue with  $\text{H}_2\text{O}$  and add directly to filtrate. Finally rinse dish with a few ml of hot HCl followed by hot  $\text{H}_2\text{O}$ .

## 15

## NOTES ON ISOLATION OF LEAD

Procedure 16, while rapid and convenient, is limited to those materials which, with the aid of citric acid, will yield the clear ammoniacal soln demanded for quantitative extraction of Pb with dithizone. Lead is readily occluded by many alkaline precipitates (Mg and Ca phosphates, Al and ferric hydroxides and silicates). Many food materials may be handled in this way as the naturally occurring amounts of these substances are not excessive. However, some materials contain more of these substances than can be kept in soln under alkaline conditions with any reasonable amount of citric acid. In these cases proceed as directed under 17. The difficulty of ammoniacal precipitation may sometimes be overcome by limiting the sample size in those cases where sampling is no problem.

## 16

## DITHIZONE EXTRACTION

(Applicable to most carbohydrates and cereal foods, fruit and fruit products, milk, fresh vegetables, plant materials, etc.)

Transfer the ash soln to 300 ml short-stemmed separatory funnel and add citric acid reagent, 13(d), equivalent to 10 g of citric acid. Make slightly alkaline to litmus with  $\text{NH}_4\text{OH}$ , keeping soln cool, and allow to stand 2–5 min. If precipitate forms, redissolve with HCl and isolate the Pb as directed under 17. If no precipitate forms, add 5 ml of 10% KCN or NaCN soln (more may be necessary if large quantities of Zn, Cu, Cd, etc., are present) and check the pH of the soln by adding a drop of thymol blue and observing color of the drop. (The pH should be 8.5 or above, blue-green to blue with thymol blue.) If ash was highly colored with Fe, keep the pH of the soln comparatively low, because a pH of 10 or above in presence of Fe may cause oxidation of the dithizone. Immediately extract with 20 ml portions of the dithizone reagent, using the weaker solns unless exceptionally large quantities of Pb are present. Shake 10–15 seconds, allow layers to separate, and note color of the  $\text{CHCl}_3$  phase. (The Pb dithizone complex is red, but the color may be masked by excess green dithizone, giving intermediate hues of purple and crimson. The color of the  $\text{CHCl}_3$  extract gives the first indication of amount of Pb present, and the progress of extraction can be followed by noting color of successive extracts.)

(a) If the Pb is to be determined electrolytically ( $\text{Pb} > 0.05 \text{ mg}$ ), draw off  $\text{CHCl}_3$  layer into 125 ml short-stemmed separatory funnel containing 25–30 ml of  $\text{H}_2\text{O}$  made ammoniacal with one drop of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90). Continue extraction until two successive extracts with small portions of the weaker dithizone solns show the negative green (not bluish or purple) color, combining extracts in smaller separa-

tory funnel. Shake, allow layers to separate, draw  $\text{CHCl}_3$  fraction into another small separatory funnel, and repeat washing process as before. Draw off  $\text{CHCl}_3$  fraction as cleanly as possible into 100 or 150 ml beaker, and pass small portion of dilute dithizone soln thru funnels in succession so as to wash out small portions of extract persisting in aqueous fraction. Add to beaker and evaporate  $\text{CHCl}_3$  with gentle heat on steam bath. Take up dry residue with 3-4 ml of  $\text{HNO}_3$ , and heat by swirling over low flame. Dilute to ca 25 ml and continue heating 1-2 min. in order to fume off oxides of N. Add small piece of litmus paper, neutralize with  $\text{NH}_4\text{OH}$ , dilute nearly to capacity of beaker, and add 1 ml of water-white  $\text{HNO}_3$  per 100 ml of soln. Proceed as directed under 19 and 20.

*Alternative procedure.*—Draw off washed  $\text{CHCl}_3$  into separatory funnel containing 110 ml of 1%  $\text{HNO}_3$ , 13(b). Shake vigorously for 1 min. to decompose lead dithizonate and draw off green  $\text{CHCl}_3$  soln. Filter acid soln thru dry filter and pipet 100 ml aliquot into 150 ml beaker. Proceed immediately as directed under 19 and 20, taking care to heat and stir the soln and to volatilize dissolved  $\text{CHCl}_3$  before adding  $\text{K}_2\text{Cr}_2\text{O}_7$  and closing electrolytic circuit. Multiply results by factor 1.1.<sup>4</sup>

(b) *If the Pb is to be determined by colorimetric dithizone procedure* ( $\text{Pb} < 0.2$  mg), do not wash the dithizone extracts with the dilute  $\text{NH}_4\text{OH}$ , but run directly into smaller separatory funnel containing 25 ml of the 1%  $\text{HNO}_3$ , 13(b). When extraction is complete, shake combined extracts in smaller separatory funnel and draw off green dithizone layer into another separatory funnel containing a further 25 ml portion of 1%  $\text{HNO}_3$ . Shake, allow layers to separate, and discard  $\text{CHCl}_3$  fraction. Filter acid extracts containing Pb in succession thru small pledget of wet cotton inserted in stem of small funnel, into 50 ml flask or glass-stoppered cylinder, using second acid extract to wash out funnel in which the first acid extraction was made. (This procedure removes  $\text{CHCl}_3$  globules.) Make up any slight deficiency in volume with the 1%  $\text{HNO}_3$  and mix. Proceed as directed under 22 and 23.

## 17

### *Sulfide Separation*

(Applicable to all products and usually necessary in the case of cacao products, tea, sardines, and all food products containing high proportion of alkaline earth phosphates, especially those of Mg, which promote formation of precipitates in ammoniacal citrate solns.)

Cool the acid soln of the ash, add citric acid reagent, 13(d), equivalent to 10 g of citric acid, and adjust to pH of 3.0-3.4 (bromophenol blue) with  $\text{NH}_4\text{OH}$ . If enough Fe is present to color soln strongly, make final adjustment with help of spot plate. (Phosphates precipitated by local action of  $\text{NH}_4\text{OH}$  may usually be redissolved by shaking and cooling.) If amount of Pb is small, add 5-10 mg of pure  $\text{CuSO}_4$  to soln to act as coprecipitant. Precipitate sulfides by passing in  $\text{H}_2\text{S}$  until soln is saturated (3-5 min.). Immediately filter with suction into flask in a bell jar (fritted glass filter, Jena 11G4 or equivalent, is preferred).

(a) *If the Pb is to be determined electrolytically* ( $\text{Pb} > 0.05$  mg), wash flask and precipitate with a few small portions of 3%  $\text{Na}_2\text{SO}_4$  adjusted to pH 3.0-3.4 and saturated with  $\text{H}_2\text{S}$ . If a clean sulfide precipitate has been obtained, dissolve sulfides with 5 ml of hot  $\text{HNO}_3$ , wetting all portions of filter; allow to stand a few minutes and draw thru into flask in which sulfide precipitation was made. Wash the filter with several portions of hot  $\text{H}_2\text{O}$ , stopper flask, shake, and boil for a few minutes to remove traces of  $\text{H}_2\text{S}$ . Cool, adjust acidity to 1% with  $\text{HNO}_3$  in 100-125 ml volume and proceed as directed in 19 and 20. If there is possibility of the sulfide precipitate being contaminated with more than 3 mg of Cl, 20 mg of  $\text{As}_2\text{O}_3$ , 30 mg of  $\text{P}_2\text{O}_5$ , 50 mg of Hg, or with  $\text{Sb}_2\text{S}_3$ , dissolve as directed above with  $\text{HNO}_3$  (without previous washing with  $\text{Na}_2\text{SO}_4$  soln), wash filter with hot  $\text{H}_2\text{O}$ , and boil soln as

before. Transfer to 200 ml separatory funnel, add the citric acid reagent equivalent to 5 g of citric acid, make ammoniacal, extract with dithizone soln, and determine Pb as directed under 16, 16(a), 19, and 20.

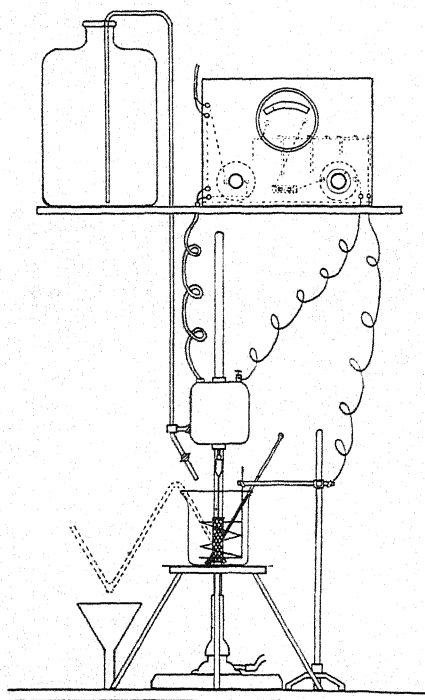


FIG. 39.—APPARATUS FOR DETERMINATION OF LEAD BY ELECTROLYTIC METHOD

anode, 1"  $\times$  5/16" and 4" over-all length, and a cathode of 18-gage Pt wire wound in spiral form. For larger amounts of Pb (over 5 mg) a cylindrical anode 2"  $\times$  1/2" is convenient.

## 19

### Electrolysis

Immediately before electrolyzing bring anode to red heat in oxidizing flame of burner. (A variable titration blank is obtained if anode is not heated just before determination, due possibly to film of oxygen adsorbed on anode and activated during electrolysis. Heating reduces and renders constant this "oxygen blank." With small anode it will be 0.07–0.1 ml of 0.001 *N* thiosulfate and with larger electrode proportionately larger. The blank for a particular anode should be determined from the average of a series of determinations conducted on pure reagents.)

In all determinations the sample at this point is contained in a volume of 100–125 ml of 1%  $\text{HNO}_3$  (with the large anode a volume of 200 ml is convenient). Place beaker (100–150 ml for small and 250 ml for large anode) in position, making sure electrodes are well covered with soln, and start motor. Heat to 60–70°, and add ca 100 mg of  $\text{K}_2\text{Cr}_2\text{O}_7$  to keep soln in oxidized state and repress formation of nitrites,

(b) If the Pb is to be determined by the colorimetric dithizone procedure (Pb < 0.2 mg), dissolve sulfides, without previous washing, with 5 ml of hot  $\text{HNO}_3$ , drawing soln thru into original flask; wash with hot  $\text{H}_2\text{O}$ , stopper, shake, and boil to remove  $\text{H}_2\text{S}$ . Transfer to 200 ml separatory funnel, add citric acid equivalent to 5 g of citric acid, make ammoniacal, extract, and determine Pb as directed under 16, 16(b), 22, and 23.

### Electrolytic Determination

(Pb 0.05–10.00 mg)

## 18

### Apparatus

See Fig. 39. Four dry cells in series constitute a convenient source of current. The meter (0–500 milliamperes), switch, fuse, rheostat (60 ohm radio type), and variable resistance for control of the motor speed (25–500 ohm, 1/2 amp. capacity) may be conveniently mounted upon a panel. The motor for rotating the anode (1/20 H.P., 110 v. universal) is equipped with a chuck and binding post. The rate of rotation should be sufficient to produce efficient circulation and may vary from 400 to 800 r.p.m. The electrodes consist of a 45-mesh, sand-blasted, Pt gauze cylindrical

especially when organic matter is present. Start current and electrolyze with ca 75 milliamperes for 20 min. at 70–80°. Use 100–150 milliamperes for larger anode. Remove flame, insert siphon in beaker, and start stream of distilled H<sub>2</sub>O playing directly on anode. Start siphon, taking care to keep level of liquid above deposit. (A convenient siphon can also be made by connecting an inverted V-shaped tube to an ordinary water-pump.) The acid is entirely removed when current falls to zero. Turn off motor, electrolytic current, and rinse water; remove anode from the chuck and give it a final rinse with H<sub>2</sub>O.

20

*Titration of PbO<sub>2</sub>*

Dissolve deposit in 4–5 ml of the “stripping” reagent, 13(f), +1 ml of the KI reagent, 13(g), contained in flat-bottomed vial of such size that soln just covers anode. Add a few drops of the starch soln, 13(h), and titrate liberated I with 0.001 *N* thiosulfate, 13(i), in the vial, using anode as stirrer and sighting down thru vial, as thru miniature Nessler tube, to detect the delicate end point. (If quantity of Pb is seen to be large (1–5 mg), use 0.005 *N* thiosulfate and double the amount of the reagents 13(f) and (g). With the 2" anode still larger amounts may be used.) No yellow insoluble PbI<sub>2</sub> should form as the deposit is “stripped”; if it does add more of the Na acetate. The deposit should dissolve completely and almost immediately. To determine amount of Pb, subtract anode and reagent blanks from the total titer and multiply by factor of the thiosulfate, 13(i).  $\text{PbO}_2 + 4\text{HI} = \text{I}_2 + \text{PbI}_2 + 2\text{H}_2\text{O}$ . The absence of interfering Bi may be assured by applying test 26(c).

21

COLORIMETRIC DITHIZONE DETERMINATION<sup>3</sup>

(Pb 0.001–0.200 mg)

The limiting factor in the determination of minute quantities of Pb by the colorimetric dithizone procedure is probably the size of the reagent blank. The importance of careful blank determinations must be especially stressed when quantities of Pb of the order of 1–5 $\gamma$  (1 $\gamma$ =0.001 mg) are being determined. With special care in purification of reagents and by the use of carefully cleaned Pyrex ware, including separatory funnels, it should be possible to reduce reagent blank to 1 $\gamma$  or possibly below. Owing to Pb-bearing dust, vapors, etc., it is necessary to expose the blank determination in muffle or on steam bath for same length of time as sample is exposed, and to use exactly same amounts of reagents (even H<sub>2</sub>O) for the blank and actual determinations.

Pb is extracted from aqueous soln, under standard conditions of volume and pH, with a definite volume of a CHCl<sub>3</sub> soln of dithizone of standard strength. The optimum pH of operation is 9.5–10.0. Dithizone strengths are so chosen that an excess of dithizone is always present in the reaction mixture. Lead is brought into the CHCl<sub>3</sub> phase in the form of the red complex, and the uncombined green dithizone partitions between the aqueous and CHCl<sub>3</sub> phases and modifies the color of the extract according to the relative amounts of Pb and dithizone. Thus, according to this proportion, a series of colors from red to green may be arranged with intermediate crimsons, purples, and blues. The volumes and strengths of the CHCl<sub>3</sub> solns depend upon the Pb range it is desired to cover and are so chosen as to give the same general color progression from red to green for each range. Limiting the range increases accuracy at the expense of flexibility. The colors produced with standard amounts of Pb furnish by comparison the basis for a quantitative estimation. The volumes and concentrations of standard dithizone for various ranges are as follows:

Pb RANGES micrograms (0.001 mg)	CONCENTRATION mg/liter	VOLUME ml	CELL LENGTH inches
0-5	4	5	2
0-10	4	10	2
0-20	8	10	1
0-50	8	25	1
0-100	10	30	$1\frac{1}{2}$
0-200	20	30	$\frac{1}{2}$

## 22

*Simple Color Matching*

Prepare 10 standards covering in equal steps the range in which it is desired to work, as follows: Use a standard Pb soln, 13(a), in 1%  $\text{HNO}_3$ , 1 ml of which equals some simple fraction or multiple of  $1\gamma$  of Pb. Measure the amounts representing the various steps of the range into a series of separatory funnels and add the pure 1%  $\text{HNO}_3$  so that total volume is always 50 ml. (It is best to add the acid first so that the Pb soln is not lost around the stopcock of the separatory funnel.) Add 10 ml of the ammonia-cyanide mixture, 13(j), and mix. The resultant pH will be ca 9.7. Immediately add the appropriate volume of standard dithizone, which depends upon range to be covered (see table), and shake for 1 min. Draw off lower layers into series of tubes or vials and arrange in order. For lower ranges, i.e., up to  $20\gamma$  of Pb, matching is best done by viewing longitudinally in small flat-bottomed vials ca 3" in length. For higher ranges, 0-50 $\gamma$  and above, depth of column must be reduced, and matching is conveniently done by viewing transversely in Nessler tubes of matched diameter, because even pure dithizone solns appear red by transmitted light if concentration or depth of column is increased beyond a certain point. If standards are kept covered when not in use they should last at least one day.

For the determination, place aliquot part, or entire amount, of the 50 ml of 1%  $\text{HNO}_3$  in which the Pb has been isolated, 16(b) and 17(b), in a separatory funnel, and if aliquot is taken, make to 50 ml with the 1%  $\text{HNO}_3$ . Add 10 ml of the ammonia-cyanide mixture, 13(j), and mix. Immediately develop the color by shaking 1 min. with proper amount of the standard dithizone. Draw off lower layer into tube or vial similar to those used with standards and compare. If range is exceeded, repeat with smaller aliquot, or re-extract with excess dithizone before draining from funnel, isolate once more in 50 ml of the  $\text{HNO}_3$  reagent, and compare with standards covering a higher range. Interpolation between steps of the various ranges should be easily made. If an aliquot of the 50 ml of the 1%  $\text{HNO}_3$  in which the Pb had been isolated is taken, subtract only a corresponding amount of the total reagent blank from amount of Pb found.

## 23

*Photometric Methods*

Transmission spectra of the two components in the dithizone extract (Pb dithizone complex and the free dithizone) show a marked difference in their ability to absorb light of wave length  $510\text{ m}\mu$ , the red Pb complex absorbing strongly and the free green dithizone transmitting freely. Thus, when the absorption of light of this wave length by the individuals of a standard color series, measured thru suitable cell-length, is determined photometrically, a practically linear relation is observed between amounts of Pb and absorption coefficient ( $-\log$  transmittancy). In making the measurements a spectrophotometer set at this wave length or a simple photometer equipped with a blue-green filter centered at about this point can be used. The dithizone solns are standardized once only with known amounts of Pb, and the labor of repeated standard preparation is necessary only when changes caused by evaporation or oxidation occur.

Standardize dithizone solns as follows: Using appropriate volumes and concentrations of solns specified for various ranges (see above) in separatory funnels, prepare standard colors as in the visual color-matching procedure, saturating the standard Pb and the 1%  $\text{HNO}_3$  solns with clear  $\text{CHCl}_3$  before use, and thereby eliminating differences in volume of extract between standards and unknowns. (It is unnecessary to prepare the full 10 steps of the range, and the number of standards may be limited to 5 or 6.) Develop the colors by shaking the funnels 1 min., allow to stand a few minutes, and filter extracts thru specially prepared filter papers (9 cm quantitative filters soaked overnight in 1%  $\text{HNO}_3$  and washed with large volumes of  $\text{H}_2\text{O}$  on Büchner funnel to remove the slight trace of acid and/or Pb usually present on even the best grades of filter paper. Fitting a 9 cm filter directly into mouth of a 50 ml low-form Pyrex beaker eliminates the use of a funnel in the filtering operation). Fill a cell of proper length with the filtered extracts for the various Pb ranges, as indicated in the table given previously, using the specified volume and strength of standard dithizone solns. Use the same all glass cell (preferably Pyrex) with plane parallel fused ends for all standards.

Determine *absorption coefficients* for the various steps of the range and plot against the quantity of Pb to obtain a standardization curve for the particular lot of dithizone. Preferably calculate the slope of the line connecting the standard points and the intercept of the line on the Pb axis, making the calculation as follows: Take the equation of the line connecting the standard points as  $X = a + bY$ , and let  $X = \gamma$  of Pb and  $Y = \text{absorption coefficient}$ ;  $a$  then represents the intercept on the Pb axis (in this case a negative value) and  $b$  represents the tangent or slope of the line. Calculate  $a$  and  $b$  from the following formula, where  $n = \text{No. of observations, including that for 0 lead, and } \Sigma \text{ represents merely "the sum."}$

$$b = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\Sigma Y^2 - \frac{\Sigma Y \Sigma Y}{n}}, \quad \text{and} \quad a = \frac{\Sigma X}{n} - b \frac{\Sigma Y}{n}.$$

Then the procedure for determining the Pb content of an unknown falling within the range is to determine the value of the absorption coefficient, using the standard dithizone and the same cell with which the standard readings were made, and calculate the Pb from the equation,  $X = a + bY$ , using the values of  $a$  and  $b$  as determined above. If protected from evaporation and direct sunlight the standard factors of dithizone solns should not change appreciably for at least a month.

For the actual determination proceed as directed in 22, except to filter the extract before photometric measurement thru the prepared filter papers. Determine the absorption coefficient, using the standardized dithizone with the same cell used in making the standard curve, and read the amount of Pb from this standard curve or calculate from the factor of the dithizone soln. If the range is exceeded, repeat with a smaller aliquot, or re-extract and repeat with dithizone standardized to cover a higher range. If an aliquot of the 50 ml of 1%  $\text{HNO}_3$  in which the Pb has been isolated is taken, subtract only a corresponding amount of the total reagent blank from the amount of Pb found.

## 24

## INTERFERENCES

If present in excessive quantities in the final determination, Cl,  $\text{P}_2\text{O}_5$ , As, Se, Te, Hg, and Bi (>5 mg) will prevent the complete electrolytic deposition of Pb; and Bi (<2 mg), Sn, Sb, Mn, and Ag will contaminate the deposit. Certain reducing agents, such as nitrites, likewise prevent complete deposition of the Pb. The genera

method leading up to the final determination of Pb by the electrolytic procedure has been so formulated that all interferences except those of Sn and Bi are eliminated. Special directions, applicable to both electrolytic and colorimetric methods, for removal of Sn and Bi are given in 25 and 26. As much as 3 mg of Cl, 20 mg of  $\text{As}_2\text{O}_3$ , 30 mg of  $\text{P}_2\text{O}_5$ , and 50 mg of Hg will not interfere in the final electrolysis, and if there is suspicion that greater quantities are present in the sulfide mixture, 17, they can be eliminated by a dithizone extraction. Interferences in the colorimetric dithizone method are limited by the use of KCN to stannous Sn, Bi, and Tl. The rarity of Tl makes its interference unlikely in ordinary work, and no method of removal is given. Dithizone itself is destroyed by strong oxidizing agents, such as free halogens and large quantities of ferric Fe in citrate-ammonia-cyanide solns, which may become troublesome in dithizone extractions.

## 25

## REMOVAL OF TIN

Tin becomes a problem in the analysis of canned foods, and in quantities above 150 p.p.m. it will usually appear in the ash soln as a milky suspension of  $\text{SnO}_2$ . It must be dissolved to facilitate filtration and to release occluded Pb. Quantities of Sn of this order may cause trouble by precipitating under the conditions of the dithizone extraction of Pb, 16.

Two procedures for elimination of larger quantities of Sn are given: (a) volatilization as  $\text{SnBr}_4$  from the acid soln of the ash, and (b) leaching the mixed sulfides with warm Na polysulfide, when the sulfide method of isolation, 17, has been applied. These procedures may not eliminate Sn completely, but the quantity should be reduced to below that necessary to interfere with the electrolytic determination of Pb. Stannic Sn is not extracted with dithizone, and as small quantities of residual Sn will be in the stannic form after application of either (a) or (b), final isolation of Pb by means of a dithizone extraction will result in *complete* removal of Sn.

In general, quantities of Sn under 50 mg should not interfere in either the electrolytic or colorimetric dithizone methods of Pb determination provided the Sn is in stannic form and a preliminary isolation with dithizone is made; hence, this method of isolation should be applied wherever possible.

(a) *Volatilization as  $\text{SnBr}_4$  from the acid soln of the ash.*—After a practically carbon-free ash has been obtained, 14, add 15–20 ml of 40% redistilled HBr. If nitrates have been used as ash aids, cover casserole with watch-glass and heat on steam bath until Br evolution diminishes, then rinse off watch-glass with  $\text{H}_2\text{O}$  and bring to boil to complete expulsion of Br. (This process destroys undecomposed nitrates.) Add more HBr, if necessary, to dissolve the ash and examine the solns for clearness. If there is an insoluble residue of  $\text{SnO}_2$ , add 50–100 mg of pure Sn, 13(k), to the simmering HBr soln of the ash and allow it to dissolve. (Metallic Sn seems to be the best agent to bring ignited  $\text{SnO}_2$  into soln. To be effective the ash soln must be in the reduced state. Ferric oxide sometimes becomes “noble” during ashing and dissolves with difficulty, but treatment with metallic Sn also brings it into soln. Treatment with Sn will be necessary only with the contents of badly corroded cans.) When soln of the ash is free from milkiness due to  $\text{SnO}_2$ , add 20 ml of 60%  $\text{HClO}_4$  (double distilled preferred), oxidize mixture with a few ml of the HBr- $\text{Br}_2$  mixture, 13(l), and then add a further 15 ml of the reagent, portion-wise, while the soln is evaporated to incipient fumes of  $\text{HClO}_4$  (ca  $150^\circ$ ) on hot plate. Repeat with another 10 ml portion of the HBr- $\text{Br}_2$  mixture if more than 100 mg of tin has been used to dissolve the ash. (Hot  $\text{HClO}_4$  helps keep the ash salts in soln and with Br holds the Sn in the volatile  $\text{SnBr}_4$  combination.) When the Sn, HBr, and Br have been completely volatilized, cool, and take up with hot  $\text{H}_2\text{O}$  (200 ml may be necessary if much  $\text{KClO}_4$  is present). Filter off any small quantities of dehydrated silica,

extract residue twice with 5 ml of the hot HCl-citric acid reagent, 13(n), and hot H<sub>2</sub>O, treat dish if necessary with NaOH as directed in 14, and isolate the Pb by dithizone extraction as directed under 16, or by sulfide separation, 17, finally determining Pb as directed under 16, 17, 19, 20, 22, and 23.

(b) *With sodium polysulfide*.—(Recommended for routine work on canned foods by the electrolytic method when Pb > 0.05 mg.)

Isolate the Pb by means of a sulfide precipitation, 17, filter, and wash flask and filter with 3–6 portions of ca 5 ml each of the warm Na polysulfide, 13(m). (Sn, As, and Sb sulfides are dissolved; CuS may be partially dissolved and reprecipitated in the filtrate.) Wash flask and residual sulfides several times with 3% Na<sub>2</sub>SO<sub>4</sub> adjusted to pH 3.0–3.4 and saturated with H<sub>2</sub>S, and proceed as directed under 17(a), beginning “dissolve sulfides with 5 ml of hot HNO<sub>3</sub>,” and continuing directly to electrolytic determination, 19 and 20. [When ash contains much Sn, as when metallic Sn has been added to dissolve insoluble metallic oxides, the sulfide precipitate will be so bulky as to be difficult to handle, and it will be necessary to use the volatilization procedure, (a).] Extract HNO<sub>3</sub> soln of dissolved sulfides and proceed as directed under 17(b), 22, and 23 for the colorimetric dithizone determination of Pb.

## 26

## DETECTION AND REMOVAL OF BISMUTH

(a) *By dithizone at pH 2.0 after preliminary dithizone extraction at pH 8–11.5*.—(This procedure completely removes small quantities of Bi and stannous Sn.)

Extract the metals from the CHCl<sub>3</sub> dithizone extract with 50 ml of 1% HNO<sub>3</sub> as directed in 16(b). Adjust acid extract to pH 2.0 (metacresol purple indicator) with 5% NH<sub>4</sub>OH and shake vigorously ca 1 min. with 10 ml CHCl<sub>3</sub> soln of dithizone containing 200–250 mg per liter. Allow layers to separate, and if CHCl<sub>3</sub> extract is orange red to red (Bi), draw it off and extract with another 10 ml portion of the dithizone soln. If shades of green or purple are visible, indicating excess of dithizone draw off CHCl<sub>3</sub> extract and extract aqueous phase once more with 5 ml of the dithizone soln (shaking should be prolonged, 3–5 min., to insure complete extraction of Bi). Continue extractions until dithizone extract remains pure green. Adjust pH of aqueous soln to 8.5 with NH<sub>4</sub>OH, add KCN, and extract with dithizone as directed in 16. Determine Pb colorimetrically as directed in 16(b), 22, and 23, or electrolytically, 16(a), 19, and 20 when the Pb > 0.05 mg.

(b) *From acid soln of sulfides*.—(Intended for small quantities of Bi, particularly when sulfide separations may be necessary.) Dissolve mixed sulfides, 17, with hot HNO<sub>3</sub> and separate Bi and Pb as directed in (a).

*Special conditions*.—(Intended for products containing large quantities of Bi.) Dissolve inorganic Bi compounds directly in HBr-Br<sub>2</sub>, 13(l). Prepare organic Bi compounds or Bi preparations mixed with organic matter containing little ash, as directed in 14, and dissolve residue in HBr-Br<sub>2</sub>. If sample contains organic matter with appreciable ash material other than Bi compounds, proceed as directed in 14 or 28, apply sulfide separation, 17, and dissolve mixed sulfides in HNO<sub>3</sub>. Evaporate HNO<sub>3</sub> soln of sulfides to dryness in porcelain dish and treat with small portions of HBr-Br<sub>2</sub> mixture. Evaporate contents of dish containing Bi dissolved in HBr-Br<sub>2</sub>, after any of the above three methods of preparation, on steam bath to volatilize Sn and to convert other metals to bromides. Evaporate to dryness and place in temp.-controlled muffle and raise temp. gradually to 300°. (AsBr<sub>3</sub> and SbBr<sub>3</sub> will volatilize first at 100° or above; BiBr<sub>3</sub> will volatilize in dense orange fumes when temp. reaches 300°.) At end of 5 min. or when fumes are no longer evolved, remove dish, cool, and treat again with small portions of HBr-Br<sub>2</sub>. Again evaporate to dryness and heat for an additional period of 5 min. at 300–325° (PbBr<sub>2</sub> does not volatilize appreciably below 350°). Remove dish, cool, and dissolve residue in hot HNO<sub>3</sub>.



Proceed with removal of last traces of Bi at pH 2.0 and determine Pb as directed in (a).

(c) *After PbO<sub>2</sub> titration in electrolytic method.*—Add to the soln from 20 in the titrating vial 0.25 g of solid KI and ca 0.5 ml of HCl. Shake, and add only sufficient Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln to discharge any starch iodide color. A pure yellow color shows the presence of the double bismuth iodide. (Under the conditions of the test, there is no interfering Cu, ferric Fe, or Sb, and 0.005 mg of Bi will show the yellow color test.) If the test is positive, reject the Pb results and repeat determination, giving special attention to removal of the Bi interference.

#### SPECIAL METHODS OF SAMPLE PREPARATION

27

##### *Solution in Acids*

(Applicable to chemicals soluble in H<sub>2</sub>O or acid, e.g., phosphates, sulfates, etc., and organic products of the type of tartrates and citrates.)

Dissolve 5–100 g of sample in HCl in 400 ml beaker, gaging amount of sample according to its nature and the amount of Pb expected. With calcium phosphates use 10–50 g. Dissolve in smallest practicable volume of soln by warming and adding alternately small quantities of hot H<sub>2</sub>O and HCl. Filter soln with suction (fritted glass filter preferred) into beaker or flask under bell jar and leach any residue with 10–25 ml of the hot HCl-citric acid, 13(n), followed by 10–25 ml of hot 40% NH<sub>4</sub> acetate. Rinse beaker and filter with hot H<sub>2</sub>O and cool soln.

Proceed as directed under 16. If interference by precipitate formation occurs, reacidify and isolate Pb by the sulfide precipitation, 17. If difficulty is experienced in obtaining a clear soln with Ca phosphates at pH 3.0–3.4 (sulfide precipitate may be contaminated with excessive phosphates), redissolve precipitate, add more citric acid soln, 13(d), readjust pH, and reprecipitate the sulfides; or make one sulfide precipitation, dissolve the sulfides in hot HNO<sub>3</sub>, boil off H<sub>2</sub>S, and extract the Pb with dithizone, 16. Sometimes difficulty due to precipitate formation in 16 can be obviated by the use of a smaller sample for extraction and colorimetric determination. If Sn or Bi is suspected, remove by methods described under 25 and 26. Finally determine the isolated Pb electrolytically, 19 and 20, or colorimetrically, 22 and 23.

28

##### *Complete Digestion*

(Applicable to most food or biological products, except fats and oils, oily products, etc.)

Digest a representative sample in Kjeldahl flask as directed under 3. Distil the arsenic if desired according to the tentative bromate method, 9. If the arsenic is not to be distilled, add 100 ml of H<sub>2</sub>O and sufficient HCl to the residue in the flask to dissolve any CaSO<sub>4</sub> that may be present. Filter on a fritted glass filter, pulverizing any insoluble residue with a flattened stirring rod (anhydrous silica or BaSO<sub>4</sub>). Dissolve any Pb sulfate in flask and leach residue on filter with 10–20 ml of the hot HCl-citric acid soln, 13(n), followed by 10–20 ml of hot 40% NH<sub>4</sub> acetate soln. Finally rinse both flask and filter with hot H<sub>2</sub>O. Isolate the Pb by the dithizone, 16, or sulfide precipitation, 17, methods. (In general, the sulfide method is preferable especially when Ba or excessive Ca sulfates are present, as insoluble sulfates readily occlude Pb.) If Bi and Sn are present, remove them as directed in 25 and 26. After isolation determine Pb according to the electrolytic, 19 and 20, or colorimetric method, 22 and 23.

29

##### *Partial Digestion or "Mush"*

(a) *For fruits or vegetables that can be peeled.*—Weigh and peel representative

sample (10–45 apples), including stem and calyx ends with peels. Stems and sepals themselves may be excluded if desired. Transfer the peels to one or more 2000 ml tared beakers, re-weigh, and record weight of sample. Add 75–200 ml of  $\text{HNO}_3$  to each of beakers, according to weight of peel therein, and warm carefully over a gauze or on steam bath in fume hood. Stew slowly, while stirring, until initial foaming decreases. Cover beaker with watch-glass and continue heating until a smooth mixture results with little or no stringiness and a greatly diminished evolution of oxides of nitrogen (15–45 min. according to amount of sample taken). Colloids (pectin) must be sufficiently destroyed to prevent emulsification in subsequent  $\text{CHCl}_3$  extractions. Dilute with  $\text{H}_2\text{O}$ , cool, and transfer contents of the one or more beakers to a 1000 or 2000 ml volumetric flask. Make to mark, mix well, and filter. Transfer 100–250 ml of filtrate to short-stemmed separatory funnel, add citric acid, 13(d), equivalent to 5 g of citric acid, make ammoniacal (the soln will darken materially), and proceed with the dithizone extraction as directed in 16. (If soln contains much sugar, extra cyanide may be necessary and Pb should be extracted immediately. Sugar residues combine with cyanides and weaken or completely destroy “masking” effect of cyanide. If cyanide is combined in ineffective combinations, other metals, notably Zn, may be extracted.) Determine the extracted Pb electrolytically, 16(a), 19, and 20. Correct for volume occupied by insoluble matter by allowing 0.075 ml per g of peel.

(b) *For products other than fruit and vegetable peels.*—(For carbohydrate foods, fresh or canned small fruits or vegetables, jams, apple butter, etc. Sn is often present, while Bi is usually absent.)

Weigh 100–200 g of well-mixed sample into 1000–2000 ml beaker. To dry samples, add about an equal weight of  $\text{H}_2\text{O}$ , add 50–150 ml of  $\text{HNO}_3$  and “mush” mixture as directed in (a). (Duration of mashing period and quantity of  $\text{HNO}_3$  should be varied according to the product. Colloids, which induce emulsification in the dithizone extraction, should be destroyed so that a clear soln is obtained upon filtration.) Cool, transfer to 500 ml flask, mix well, and filter. Transfer 100–250 ml aliquot of filtrate to separatory funnel and proceed as directed in (a), concluding with electrolytic determination. (Interference of Sn is generally negligible.)

#### *Rapid Method Restricted to Apples and Pears—Official*

(Efficiency of 95% expected.)

(For rapid determination of Pb spray residue on apples and pears. According to convention results should be expressed as grains per lb.; grains/lb.  $\times 143$  = p.p.m.; p.p.m.  $\times .007$  = grains/lb.)

#### 30

#### PREPARATION OF SAMPLE

Weigh 10 or more apples or pears and pull or cut out stems with narrow-bladed knife so as to expose junction of stem and fruit to action of the solvent, cutting no more of flesh than necessary. Trim off sepals (dried residue of blossom) so that solvents have unimpeded entrance to and egress from calyx cup. Allow stems and sepals to fall into large funnel inserted in neck of 500 ml volumetric flask. (No harm results if stems and sepals fall into flask. If for purpose of analysis inclusion of Pb content of stems and sepals is not desired, these may be discarded.) To 25 ml of the 30% NaOH in 600 ml beaker, add 175 ml of  $\text{H}_2\text{O}$  and 25 ml of Na oleate, 13(o), and bring to gentle boil. Have ready in wash bottle 250 ml of hot  $\text{HNO}_3$  (2+98) or hot HCl (3+97). (HCl is preferred if As is to be determined later.) Impale each fruit in turn upon pointed glass rod, immerse in the alkaline soln, with occasional rotation until skin begins to check, then remove to funnel and rinse with stream of the hot

acid, being careful to flush out stem and calyx ends thoroly and to allow the rinse acid to flow over stems and sepals in funnel. When all fruit has been thus treated, cool the alkaline soln and add it thru funnel to acid soln in flask. Rinse beaker and funnel with any remaining acid and with  $H_2O$ , using entire 250 ml of rinse acid. Cool, and make to volume. In 200 ml Erlenmeyer flask place exactly 10 ml of  $HNO_3$ . Thoroly mix contents of flask and immediately add 100 ml to acid in flask while swirling vigorously. Filter on rapid filter. If first portion of filtrate is cloudy, return it to filter until clear filtrate is obtained. Determine Pb as directed in 31 or 32, or use 25 ml of acid and 250 ml of wash soln and proceed electrolytically as directed under 33..

## 31

## DETERMINATION WITH NESSLER TUBES

(At least 15 tubes matched for uniformity in color and *diameter* are necessary.)

(a) *Standards*.—Introduce into each of two 1 liter volumetric flasks 47.5 ml of 30% NaOH. When  $HNO_3$  has been used in rinsing and acidification, 30, add 100 ml of  $HNO_3$  to each flask. When HCl (3+97) has been used in rinsing, add 91 ml of  $HNO_3$  and 13.6 ml of HCl to each flask. Do not mix the acids unless solns are cold and dilute. To one of flasks add the stock reagent, 13(a), equivalent to 9.82 mg of Pb. Mark this flask "standard" and the other "blank." Dilute both solns to volume at room temp. and mix. These two solns contain the reagents as they occur in an acidified and filtered sample soln. The "standard" is equivalent in Pb content to an acidified soln from a sample of 1400 g carrying a Pb load (removable by "stripping" procedure) of 0.027 grain/lb. By a combination of the two solns in suitable proportions the equivalent of any Pb load from 0 to 0.027 grain/lb may be obtained.

The standard tubes may be made up in intervals corresponding to 0.003 grain/lb. and then interpolation to 0.001 grain/lb. is possible. The following table gives the quantities of "Standard" and "Blank" to be added to the Nessler tubes for each interval. They are conveniently measured into the tube by means of burets.

GRAIN/LB.	STANDARD <i>ml</i>	BLANK <i>ml</i>
0.000	0.0	20.0
0.003	2.2	17.8
0.006	4.5	15.5
0.009	6.7	13.3
0.012	8.9	11.1
0.015	11.1	8.9
0.018	13.3	6.7
0.021	15.5	4.5
0.024	17.8	2.2
0.027	20.0	0.0

Add to each tube 10 ml of the ammonia-cyanide-citrate soln, 13(p), followed by 25 ml of standard dithizone soln (25 mg of *purified* dithizone dissolved in 1 liter of  $CHCl_3$  and preserved in a dispensing apparatus to prevent evaporation). Shake vigorously for 1 min. and allow the layers to separate. The pH of the aqueous phase should be ca 9.4 regardless of whether HCl or  $HNO_3$  is used in rinsing. Stopper each standard tube securely with new cork stopper. It is unnecessary to make up entire series of standards if only a portion of the range, for example, 0.015–0.025 grain/lb., is of quantitative interest.

(b) *Comparison*.—Transfer 20 ml portions of the filtrate from 30 to each of three Nessler tubes. First add 10 ml of the ammonia-cyanide-citrate soln, 13(p), to each tube; to one tube add 20 ml of standard dithizone soln (see standards) and to the other two tubes 20 ml of clear  $CHCl_3$ . Shake tubes vigorously for 1 min. and allow

layers to separate. With a tube of clear  $\text{CHCl}_3$  backing the sample tube (containing the dithizone) and one sample tube containing  $\text{CHCl}_3$  backing each of two standard tubes, compare the color in the lower layer of the sample with that of the standards, looking thru tubes at right angles to their lengths toward a strong diffused light. (A comparator box similar to the boxes used in colorimetric pH measurements but of larger size will be found convenient.) When working with apple strip solns, a slight turbidity is produced in the sample tube, which slightly changes the color observed. To compensate for this effect, introduce the same turbidity in the field of view of the standard tubes made up exactly as in the sample, except that  $\text{CHCl}_3$  is substituted for the dithizone soln.

If the range is exceeded, i.e., if the color produced by the sample is redder than the 0.027 grain standard, repeat with smaller aliquot of filtrate, making up to 20 ml with the "blank" soln. If, for example, a 10 ml aliquot is taken, the indicated reading must be doubled. After a match has been obtained, calculate result to basis of 20 ml aliquot and 1400 g sample.

## 32

## DETERMINATION WITH PHOTOMETER

This procedure lends itself readily to photometric methods of measuring the "mixed color" (see 23). Changes in 31 are introduced here to prevent formation of colors too dense for measurement. Use 10 ml instead of 20 ml aliquots of the acidified wash soln, 30.

(a) *Standards*.—Measure following proportions of "standard" and "blank" solns, 31, into separatory funnels:

Grain/lb	0	0.006	0.012	0.018	0.024
Standard ml	0	2.2	4.4	6.7	8.9
Blank ml	20	17.8	15.6	13.3	11.1

Add 10 ml of the ammonia-cyanide-citrate soln, 13(p), and immediately develop the colors by shaking 1 min. with 20 ml of pure dithizone soln of 15 mg/liter strength. Allow to stand a few minutes to cool, filter the  $\text{CHCl}_3$  layers thru specially washed filter papers, 23, and fill a one-half inch cell. Determine absorption coefficients and plot against grain/lb of Pb to obtain a standard curve.

(b) *Comparison*.—Place an appropriate sized aliquot of the acidified strip soln in a separatory funnel and make up to 20 ml with the "blank" soln. Add 10 ml of the ammonia reagent, 13(p), and shake out with 20 ml of the standard dithizone soln. Allow to stand a few minutes to cool (considerable heat is developed in neutralization of the acid), filter, and read as directed above. Determine quantity of Pb from the standard curve as prepared in (a) and calculate to basis of a 10 ml aliquot and 1400 g sample.

## 33

## ELECTROLYTIC DETERMINATION OF LEAD IN APPLE FILTRATE

Transfer 200 ml of the acid filtrate to a separatory funnel, add equivalent of 5 g of citric acid, 13(d), make ammoniacal, add 5 ml of the 10% KCN soln, extract with dithizone as directed in 16(a), and finally determine the Pb electrolytically as directed in 19 and 20.

MERCURY<sup>a</sup>—TENTATIVE

(Applicable to leafy vegetables.)

## 34

## REAGENTS

(a) *Hydrogen peroxide*.—30% electrolytic soln.

(b) *Standard mercury solns*.—(1) Dissolve 500 mg of pure metal in  $\text{HNO}_3$  and

dilute to 1 liter; (2) dilute 10 ml of soln (1) to 500 ml with  $H_2O$  containing a few ml of  $HNO_3$  (1 ml = 0.01 mg of Hg).

(c) *Nitric acid*.—If 50 ml of acid shows a blank of more than 4 micrograms purify by distilling over  $H_2SO_4$  in an all-glass apparatus and adjust to standard strength.

(d) *Diphenylthiocarbazon* (dithizone).—Purify as directed under 13(e), if necessary. Prepare as follows:

(1) *Strong soln*.—Dissolve 50 mg of dithizone in  $CHCl_3$  and dilute to 100 ml.

(2) *Extraction soln*.—Dissolve 10 mg of dithizone in  $CCl_4$  and dilute to 200 ml.

(3) *Titrating soln*.—Measure 10.0 ml of soln (1) into a 500 ml volumetric flask and dilute to mark with  $CHCl_3$ .

Preserve solns in dark bottles at 5–10°.

## 35

## APPARATUS

*Digestion flask and internal condenser*.—Use 2 liter Florence flask of resistant glass, preferably Pyrex, fitted with internal condenser, a glass cylinder ca 9.5" long and ca 1.5" in diameter, enlarged at top to retain it in neck of flask. The bottom is cone-shaped and closed. The top may be closed with two-holed stopper or entire condenser may be made of glass. The condenser has an inlet tube extending nearly to the bottom, and an outlet tube. The condenser should fit closely inside neck of the Florence flask and should extend ca 1.5" into body of flask. The dimensions of condenser will vary slightly according to dimensions of flask.

## 36

## DETERMINATION

Introduce into digestion flask suitable weighed portion of the finely chopped and well-mixed sample (100–150 g in the case of lettuce). Add 50 ml of  $HNO_3$  and 200 ml of  $H_2O$ . Place the internal condenser in flask and start the  $H_2O$  flowing thru it.

Heat flask over low flame or on hot plate until contents boil, and reflux for 25 min. (In all refluxing the boiling must be gentle, so that top half of neck of flask remains cool). Remove flask from flame and cool nearly to room temp. Rinse condenser with  $H_2O$  from wash bottle and remove it. Filter contents quite rapidly thru large Büchner funnel (11–18.5 cm in diameter). Wash flask with ca 30 ml of  $H_2O$ , decanting it onto filter when nearly all soln has passed thru. Repeat washing once or twice. Return liquid to digestion flask, washing it in with small quantity of  $H_2O$  from wash bottle.

Digest as follows: Add 12–14 g of  $KMnO_4$ . (Add in several portions to cabbage or other foods that react vigorously or froth unduly; to lettuce extract it may be added at once.) Replace internal condenser, and when reaction subsides heat gently to boiling and reflux 10–15 min. Partially cool flask in bath, raise condenser, and add 10–12 g of  $KMnO_4$  as fast as vigor of reaction will permit.

Replace condenser and again heat soln to boiling 12 min. unless it clears in less time. Cool, add 9 or 10 g of  $KMnO_4$  and ca 20 ml of  $HNO_3$ , and repeat digestion and refluxing ca 15 min. Continue the cooling, addition of  $KMnO_4$ , and subsequent heating until the purple color of  $KMnO_4$  persists when liquid is heated almost to boiling. Agitate soln during heating to avoid bumping caused by accumulation of black oxides of Mn. If several more additions of  $KMnO_4$  are required, add more  $HNO_3$ . (For each 4 g of  $KMnO_4$  used 5 ml of  $HNO_3$  is required to combine with the reduced Mn and K. All but the most refractory organic matter should be oxidized, otherwise nitrites, formed by the action of  $HNO_3$  on organic matter, will decompose the dithizone, as shown by loss of color, and thus prevent complete extraction of Hg. Oxidation is sufficiently complete when supernatant liquid appears white after the  $MnO_2$  has settled out.)

Cool, and add  $\text{H}_2\text{O}_2$ , a little at a time, while shaking with rotary motion, until precipitated oxides of Mn dissolve completely. Be careful to insure an excess of  $\text{HNO}_3$ , otherwise a large quantity of peroxide may be used to no advantage. Replace condenser and heat to boiling 8 min. to remove free O and to dissolve refractory particles, then cool again. Add 0.5 g of crystallized hydroxylamine sulfate or chloride (soln should have strength of about 0.5%  $\text{HNO}_3$ ). Conduct a blank determination on the reagent used. One blank may be used for a particular batch of acid or  $\text{KMnO}_4$ , etc.

To concentrate the Hg and remove interfering substances, extract liquid in portions not to exceed 425 ml as follows:

Add 2 ml of dithizone soln (2), shake vigorously ca 15 seconds, allow the  $\text{CCl}_4$  phase to separate, and note its color. (The Hg-dithizone complex is orange yellow. The observation will be used in the determination later.) Add an additional 6 ml of the dithizone reagent (2), shake, etc., and again note color of  $\text{CCl}_4$  layer. Then add 10 ml of the reagent, shake vigorously 20–30 seconds, and after separation of liquids carefully draw off extract into clean separatory funnel (125–250 ml). If extract is still orange yellow, continue the extraction with 20 ml portions of reagent as long as the orange yellow extract is obtained. Remove extract from funnel containing the first extract each time. (The bright orange-yellow color of the Hg complex should not be confused with the slow fading of the dithizone reagent to the weak green or yellow that sometimes occurs due to oxidation.) When a green, light green, or reddish extract is obtained, extract once more with a combination of 5 ml of dithizone soln (2) and 10 ml of plain  $\text{CCl}_4$ . (The red or reddish violet color is due to copper that is extracted after the Hg and continuing the extraction will only remove more Cu.) If the last extractions are red (Cu), add to combined extracts 20 ml of dithizone soln (2) to assist in inhibiting the transfer of Cu with thiosulfate.

To the combined dithizone- $\text{CCl}_4$  extracts, etc., add 40 ml of  $\text{H}_2\text{O}$ , 1 ml of  $\text{HNO}_3$  (1+19), and 5 ml of 1%  $\text{Na}_2\text{S}_2\text{O}_3$ . Close funnel and shake vigorously 30 seconds. Allow layers to separate and draw off  $\text{CCl}_4$  layer. Filter aqueous layer thru plug of wet cotton in a short-stemmed funnel into 500 ml Erlenmeyer flask to remove any  $\text{CCl}_4$  droplets. Wash separatory funnel with two 5 ml portions of  $\text{H}_2\text{O}$  from a wash bottle and then use these portions to wash the short-stemmed funnel by allowing the  $\text{H}_2\text{O}$  to run down sides of funnel. Add 1 ml of  $\text{HNO}_3$  (1+1) and 6 ml of a saturated soln of  $\text{KMnO}_4$  to the liquid in the flask. Place rubber stopper (previously boiled in  $\text{H}_2\text{O}$ ) carrying an air condenser in flask and place it on steam bath 8–10 min.

Remove flask from steam bath and cool to approximately body temp. (33–40°). Add slowly (dropwise) a 10% soln of  $\text{NH}_2\text{OH} \cdot \text{HCl}$  while shaking with gentle rotation until soln clears. Add last few drops very slowly and rotate flask to dissolve any particles on the side. Then add 0.4–0.5 ml of  $\text{NH}_2\text{OH} \cdot \text{HCl}$  soln in excess. Place thermometer in flask and warm on steam bath to 60°. Remove flask and thermometer from bath, and cover flask for 1 min., then cool under cold running  $\text{H}_2\text{O}$ .

If quantity of Hg is greater than 250 micrograms (i.e., when more than the first 8 ml of the previous extracts were orange yellow), make soln to an exact volume and use aliquot for determination, otherwise use entire sample. Place soln to be titrated, at 22–26°, in 125 ml pear-shaped separatory funnel. Dilute to 100 ml (mark funnel), add 0.45 ml of clear  $\text{CHCl}_3$  (from 1 ml graduated pipet or buret graduated to 0.05 ml), and shake to saturate aqueous layer with  $\text{CHCl}_3$ .

*Minute quantities.*—(0–15 micrograms—i.e., first 2 ml of previous dithizone extractions was green to olive, not full orange yellow.) Add 1 ml of dithizone (10 mg per liter in  $\text{CHCl}_3$ ) and shake vigorously 20–30 seconds. When layers separate, observe color of  $\text{CHCl}_3$  layer, and if yellow, add another portion of dithizone and shake

again (allow all dithizone added to remain in funnel), continuing until an olive color indicating an excess of dithizone is obtained (not more than 5-6 ml should be required). A blue-green extract in the beginning indicates little or no Hg. Read accurately the dithizone used. In a second funnel of same size and shape as first, place 100 ml of an aqueous soln containing 1 ml of  $\text{HNO}_3$  (1+1) and 0.4 ml of 10%  $\text{NH}_2\text{OH} \cdot \text{HCl}$ . Add 0.45 ml of  $\text{CHCl}_3$ , and from the titration required for sample estimate approximate quantity of Hg it contains (4 micrograms per ml will turn the dithizone yellow). Add the estimated quantity of standard Hg soln to second funnel. Titrate with dithizone as in sample until the colors of extracts are the same, using a white background to observe them. If colors show considerable hue differences, it is probably due to incomplete elimination of Cu. The copper should then be removed by the procedure given under *Removal of Copper*. (Titration is preferably done by adding to the standard a smaller quantity of dithizone than is required by the Hg present to obtain a yellow extract and then adding more dithizone until colors are alike.) From the titrations and the Hg in the standard, calculate Hg in sample as follows.

$$\frac{A}{S} \times \text{micrograms Hg in standard} = \text{micrograms Hg in sample titrated.}$$

A = ml dithizone added to sample, and

S = ml dithizone added to standard.

*Large quantities.*—(16-250 micrograms). Add the standard dithizone soln (10 mg per liter) in 3 ml portions, shaking after each addition. Draw off extracts after each second addition if it is saturated with Hg (full orange yellow). Continue to add the dithizone soln until a dirty yellowish green extract (as compared with the saturated Hg complex) is obtained. Add sufficient standard Hg soln to produce the full orange-yellow saturated Hg-dithizone complex and add 8-10 micrograms in excess.

Measure accurately Hg soln added. Shake funnel vigorously 20 seconds to saturate the dithizone and after layers have separated draw off lower solvent layer. Filter aqueous portion thru a plug of damp cotton in a short-stemmed funnel into another separatory funnel to remove  $\text{CHCl}_3$  globules. Wash once or twice with 5 ml portions of  $\text{H}_2\text{O}$ . Determine the Hg remaining in aqueous soln by the comparative titration for small quantities previously given. Determine Hg equivalent of dithizone when fully saturated with Hg by titrating a standard in a similar manner. To obtain correct quantity of Hg in sample subtract that added at end of titration from total Hg equivalent of the dithizone used.

*Removal of copper.*—Ordinarily Cu is removed by the thiosulfate transfer, but if the sample is not rendered sufficiently free by this procedure remove as follows: Shake combined dithizone extracts with 60 ml of  $\text{H}_2\text{O}$  soln containing a few drops  $\text{H}_2\text{SO}_4$  (1+1), a few crystals of KI, and a few drops of 5% soln of Na arsenite to prevent the liberation of free I. Shake vigorously ca 20 seconds and carefully draw off dithizone layer. Wash aqueous soln with a little  $\text{CHCl}_3$ . (This treatment leaves the Cu in the extract and transfers the Hg to the aqueous phase.) Make solns slightly ammoniacal and extract with dithizone until an orange-yellow extract is no longer obtained. Transfer the Hg to an aqueous soln with acid thiosulfate as previously directed and continue determination.

#### TIN

Digest a 50-100 g sample as directed under 3.

38

*Gravimetric Method<sup>7</sup>—Tentative*

Add 200 ml of  $H_2O$  to the digested sample and transfer to 600 ml beaker. Rinse the Kjeldahl flask with 3 portions of boiling  $H_2O$ , making a total volume of ca 400 ml. Cool, and add  $NH_4OH$  until just alkaline, then 5 ml of  $HCl$  or 5 ml of  $H_2SO_4$  (1+3) for each 100 ml of soln. Place beaker, covered, on hot plate; heat to ca  $95^\circ$  and pass in slow stream of  $H_2S$  for an hour. Digest at  $95^\circ$  for an hour and allow to stand 30 min. longer.

Filter, and wash the precipitate of  $SnS$  alternately with 3 portions each of wash soln (100 ml of saturated  $NH_4$  acetate soln, 50 ml of glacial acetic acid, and 850 ml of  $H_2O$ ) and hot  $H_2O$ . Transfer filter and precipitate to 50 ml beaker, add 10–20 ml of  $NH_4$  polysulfide, IX, 1(i), heat to boiling, and filter. Repeat digestion with  $NH_4$  polysulfide and filtration twice, and wash filter with hot  $H_2O$ . Acidify combined filtrate and washings with acetic acid (1+9), digest on hot plate for an hour, allow to stand overnight, and filter thru a double 11 cm filter. Wash alternately with 2 portions each of the wash soln and hot  $H_2O$  and dry thoroly in weighed porcelain crucible. Ignite over Bunsen flame, very gently at first to burn off filter paper and to convert the sulfide to oxide; then partly cover crucible and heat strongly over large Bunsen or Meker burner. ( $SnS$  must be roasted gently to the oxide, which may be heated to high temp. without loss by volatilization.) Weigh as  $SnO_2$  and calculate to metallic  $Sn$ , using factor 0.7877.

*Volumetric Method<sup>8</sup>—Tentative*

39

## REAGENTS

(a) *Air-free wash soln.*—Dissolve 20 g of  $NaHCO_3$  in 2 liters of boiled  $H_2O$  and add 40 ml of  $HCl$ . This soln should be freshly prepared.

(b) *Iodine.*—0.01 *N*. Standardize soln frequently against (c), adding asbestos mat and proceeding as described under 40, omitting precipitation with  $H_2S$  and the boiling with  $HCl$  and  $KClO_3$ . The quantity of  $Sn$  in the soln used for standardization should equal approximately that contained in sample under examination.

(c) *Standard tin soln.*—Dissolve 1 g of  $Sn$  in ca 500 ml of  $HCl$  and dilute to 1 liter with  $H_2O$ . 1 ml contains 1 mg of  $Sn$ .

(d) *Sheet aluminum.*—About 30 gage, free from  $Sn$ .

40

## DETERMINATION

Proceed as directed under 38 to "Digest at  $95^\circ$  for an hour and allow to stand 30 min. longer."

Filter thru asbestos in Gooch crucible having a detachable bottom, using suction. Wash the precipitate of  $SnS$  a few times and transfer detachable bottom and asbestos pad to 300 ml Erlenmeyer flask. Remove all traces of precipitate from inside of crucible by means of a jet of hot  $H_2O$  and a rubber-tipped rod, using a minimum quantity of  $H_2O$  for washing.

Add to flask 100 ml of  $HCl$  and 0.5 g of  $KClO_3$ . Boil ca 15 min., making ca 4 more additions of smaller quantities of the  $KClO_3$  as  $Cl$  is boiled out of the soln. Wash the particles of  $KClO_3$  down from neck of flask with  $H_2O$  and finally boil to remove  $Cl$ . Add ca 1 g of the sheet  $Al$  to dispel last traces of  $Cl$ .

Fit a 2-holed rubber stopper to flask. Thru one of holes pass bulbed glass tube that reaches nearly to surface of liquid. Attach this tube to large  $CO_2$  generator thru scrubber containing  $H_2O$ . The  $CO_2$  passes out of flask thru short, bulbed tube inserted in second hole of rubber stopper and terminating slightly below it. Connect this second glass tube by means of rubber tube with another glass tube, ca 10" long,



which is immersed in a cylinder of  $\text{H}_2\text{O}$  to depth of ca 8". (This connection will act as a seal to restrain any strong flow of gas when not desired and to permit pressure in flask.)

Raise delivery tube nearly out of the  $\text{H}_2\text{O}$  seal, thus allowing rapid flow of  $\text{CO}_2$  for a few minutes to dispel air from system. Then lower delivery tube into  $\text{H}_2\text{O}$  seal, slightly raise stopper, and quickly drop into flask 1-2 g of the Al foil, folded into narrow bent strip to prevent breaking flask. When the Al has completely dissolved, raise tube in  $\text{H}_2\text{O}$  seal, allowing the  $\text{CO}_2$  to pass thru rapidly; place flask on hot plate and boil for a few minutes. Remove flask from heat and cool with tap or ice  $\text{H}_2\text{O}$ , continuing flow of  $\text{CO}_2$ . Lower delivery tube into cylinder, disconnect flask, and, with a glass plug, close rubber tube thru which the  $\text{CO}_2$  enters flask. Wash glass tubes, rubber stopper, and sides of flask with the air-free wash soln; add starch indicator, 13(h), and titrate immediately with the 0.01 N I soln.

If desired, make the titration by slightly raising rubber stopper after cooling and adding an excess of the 0.01 N I soln. Then disconnect flask, wash tubes, rubber stopper, and sides of flask with the air-free wash soln; and titrate the excess of I with 0.01 N  $\text{Na}_2\text{S}_2\text{O}_3$ .

#### COPPER<sup>a</sup>—VOLUMETRIC METHOD—TENTATIVE

(Minimum of 1 mg Cu.)

#### 41

##### PREPARATION OF SAMPLE

Digest a 50-100 g sample as directed under 3, or ash it as described under 14.

#### 42

##### REAGENTS

(a) *Standard copper soln.*—Dissolve 318 mg of pure metallic Cu in  $\text{HNO}_3$  and evaporate to dryness on steam bath. Add sufficient  $\text{H}_2\text{O}$  and a few drops of acetic acid to dissolve the  $\text{Cu}(\text{NO}_3)_2$  and again evaporate to dryness on steam bath. Redissolve the  $\text{Cu}(\text{NO}_3)_2$  as above and make up to 1 liter.

(b) *Sodium thiosulfate soln.*—Dissolve 24.82 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  to make an approximate 0.1 N soln. Allow to stand, preferably ca 2 weeks. Prepare 0.005 or 0.01 N solns by dilution of this reagent with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  in the ratio of 1:20 or 1:10. Standardize daily against the standard Cu soln in following manner: Place 20 ml of the standard Cu soln in 100 ml Erlenmeyer flask, add excess of  $\text{NH}_4\text{OH}$ , and continue as directed under 43, beginning "and boil gently to drive off excess ammonia." 1 ml of 0.01 N  $\text{Na}_2\text{S}_2\text{O}_3 = 0.6357$  mg of Cu.

#### 43

##### DETERMINATION

Dissolve ashed sample in  $\text{HCl}$  and neutralize this soln, or neutralize the soln obtained by the wet digestion, with  $\text{NH}_4\text{OH}$ . Add 5 ml of  $\text{H}_2\text{SO}_4$ , dilute soln to 200 ml, and boil for 1 min. Add cautiously 10 ml of a hot saturated soln of  $\text{Na}_2\text{S}_2\text{O}_3$  and continue boiling for 5 min. [With larger quantities of Cu the precipitate coagulates, and the liquid becomes practically clear. A few ml of 1%  $(\text{NH}_4)_2\text{SO}_4$  soln may be added to hasten coagulation.] Filter precipitate and wash 6 times with hot  $\text{H}_2\text{O}$ . Reserve filtrate for determination of Zn, if necessary. Fold precipitate within filter paper, place in small crucible, and ignite in electric muffle at  $500^\circ$ . Treat residue with 1 ml of  $\text{HNO}_3$  (2+5) and dry on steam bath. Add 5 ml of  $\text{H}_2\text{O}$  and again evaporate to dryness on steam bath. Add 20 ml of  $\text{H}_2\text{O}$  and an excess of  $\text{NH}_4\text{OH}$ , and heat on steam bath until Cu salts are dissolved. Transfer to 100 ml Erlenmeyer flask and boil gently to drive off excess ammonia. Make acid to litmus paper with acetic acid (1+1), add 1 ml in excess, boil soln 1 min. and cool to room

temp. Add 2 g of KI dissolved in enough  $H_2O$  to make final soln 50 ml, and titrate the free I immediately with 0.01 *N* or 0.005 *N*  $Na_2S_2O_3$  (according to amount of Cu present, as shown by degree of blue color in ammoniacal soln) until end point is nearly reached. Add 2 ml of starch soln, 13(h), and continue titration dropwise to disappearance of blue color. Compare with titrated standard.

## ZINC

*Gravimetric Method—Tentative*

(Minimum of 2 mg Zn.)

44

## REAGENTS

(a) *Sodium or ammonium acetate soln.*—Dissolve 50 g of the salt in  $H_2O$  and make up to 100 ml.

(b) *Ferric chloride soln.*—Dissolve 10 g of  $FeCl_3 \cdot 6H_2O$  in 100 ml of  $H_2O$ .

45

## DETERMINATION

Boil the filtrate containing the Zn obtained after filtering off the CuS (43) to expel  $H_2S$  and reduce volume to 250–300 ml, add a drop of methyl orange indicator and 5 g of  $NH_4Cl$ , and make alkaline with  $NH_4OH$ . Add HCl (1+9) dropwise to faintly acid reaction, then add 10–15 ml of the Na or  $NH_4$  acetate soln and pass in  $H_2S$  until precipitation is complete. Allow precipitate to settle, filter (clear filtrate is necessary), and wash precipitate twice with  $H_2S$  water. Dissolve precipitate on filter with a little HCl (1+3), wash filter with  $H_2O$ , boil combined filtrate and washings to expel  $H_2S$ , cool, and add distinct excess of Br water. Add 5 g of  $NH_4Cl$  and then  $NH_4OH$  until color of free Br disappears. Add HCl (1+3) dropwise until Br color just reappears; then add 10–15 ml of the Na or  $NH_4$  acetate soln and 0.5 ml of the  $FeCl_3$  soln, or enough to precipitate all the phosphates. Boil until all iron is precipitated. Filter while hot and wash precipitate with  $H_2O$  containing a little Na acetate. Pass  $H_2S$  into the combined filtrate and washings until all the  $ZnS$ , which should be pure white, is precipitated. Filter thru weighed Gooch crucible, previously heated to constant weight, and wash with  $H_2S$  water containing a little  $NH_4NO_3$ . Dry crucible and its contents in oven, ignite at bright red heat ( $900^\circ$ ), cool, and weigh as  $ZnO$ . Calculate weight of metallic Zn, using factor 0.8034.

*Colorimetric Method<sup>10</sup>—Tentative*(All  $H_2O$  must be redistilled from glass.)

46

## REAGENTS

(a) *Standard zinc soln.*—Dry reagent Zn (30-mesh or finer), transfer 2 g to volumetric flask, and add ca 200 ml of  $H_2O$  and gradually a slight excess of distilled HCl. Boil until soln is complete and make to 2000 ml at  $25^\circ$ . 1 ml contains 0.001 g of Zn.

(b) *Dilute zinc soln.*—To 4 ml of the standard Zn soln add sufficient  $H_2O$  at  $25^\circ$  to make 2000 ml. 1 ml contains 0.000002 g of Zn.

(c) *Hydrochloric acid.*—0.20 *N*. Distil the HCl into cold metal-free  $H_2O$  by allowing the concentrated acid to drip from a separatory funnel into hot  $H_2SO_4$  below surface, and dilute to required strength.

(d) *Ammonium hydroxide.*—0.20 *N*. Distil the  $NH_4OH$  to ca  $70^\circ$  into cold metal-free  $H_2O$  and dilute to required strength.

(e) *Sodium diethyldithiocarbamate.*—2.5 g per 1000 ml.

(f) *Diphenylthiocarbazone* (dithizone).—Dissolve 0.015 g of dithizone in 10 ml

of the  $\text{NH}_4\text{OH}$  (d), crushing the aggregates to facilitate soln, and transfer to 250 ml pear-shaped separatory funnel with 90 ml of  $\text{H}_2\text{O}$ . Shake out with 10 ml portions of  $\text{CCl}_4$  to a green color, discard solvent layers, and filter aqueous portion thru washed ashless paper. Prepare fresh soln daily. 1 ml is generally sufficient for 0.01 mg of reacting metals.

(g) *Ammonium citrate soln.*—225 g, or 210 g of Pb-free citric acid, per 2000 ml. Dissolve the  $\text{NH}_4$  citrate in  $\text{H}_2\text{O}$ , add distilled  $\text{NH}_4\text{OH}$  until sharply alkaline to litmus, and make to volume. Transfer 250 ml to 750 ml pear-shaped separatory funnel, add an excess of dithizone (usually 3, 2, and 1 ml, respectively), and shake out with three 25 ml portions of  $\text{CCl}_4$  to a green color. Discard solvent layers and filter aqueous portions thru washed ashless paper.

(h) *Potassium cyanide soln.*—10% (50 g per 500 ml).

(i) *Carbon tetrachloride.*—Redistilled.

As the reagents and the ash solns are vitiated by standing in contact with glass for any length of time, probably due to absorption of Zn or possibly Pb, prepare and purify them frequently. Purified or synthetic ceresin, paraffin, and other wax mixtures have been suggested as protective coatings for the inside of the reagent bottles, and if found to adhere at laboratory temperature may prevent contamination of the solutions. Pyrex glass is recommended.

## 47

## DETERMINATION

Transfer 4 g of finely ground (1 mm) air-dry material to flat-bottomed Pt dish and calcine to white or gray ash in electric muffle at temp. not exceeding visible redness. Pulverize with agate pestle and reheat if necessary to destroy carbon particles. Transfer powdered ash to 100 ml volumetric flask with small portions of  $\text{H}_2\text{O}$  and a policeman if needed. Add distilled  $\text{HCl}$  drop-wise until mixture is faintly acid to litmus and then add 20 ml of the 0.20  $N$   $\text{HCl}$  in excess. Heat to boiling to insure complete solution of metals, cool, make to volume, and filter thru dry ashless paper.

Pipet 10 ml (0.40 g) of the ash soln (size of aliquot may be varied by analyst, depending upon whether a light color from a small aliquot or a darker shade from larger quantities is preferred) into 250 ml glass-stoppered separatory funnel (pear-shaped with short delivery tube), add 20 ml of  $\text{H}_2\text{O}$ , 7 ml (2 ml to neutralize and 5 ml to produce 0.20  $N$  soln) of the  $\text{NH}_4\text{OH}$  soln, 10 ml of the  $\text{NH}_4$  citrate soln, 10 ml of  $\text{CCl}_4$ , and sufficient dithizone reagent in small portions to impart a yellow color to the aqueous soln. Shake vigorously at least 2 min. to extract Cu, Pb, and Zn (also Co, Cd, and Hg when present). Allow mixture to separate and draw off solvent layer into second separatory funnel. Discard the ammoniacal aqueous portion, which should be slightly yellow and contain the non-reacting bases and acids.

To the  $\text{CCl}_4$  layer add 45 ml of  $\text{H}_2\text{O}$  and 5 ml of the  $\text{HCl}$  soln and shake out to isolate the Cu, removing the Pb and Zn as chlorides in the acid aqueous soln, which should be colorless. Draw off solvent layer and discard unless a determination of Cu is desired.

To acid aqueous layer, add 19 ml of  $\text{H}_2\text{O}$ , 15 ml of the  $\text{NH}_4\text{OH}$  soln, 10 ml of the  $\text{NH}_4$  citrate soln, 10 ml of the  $\text{CCl}_4$ , 5 ml of the carbamate reagent, and sufficient dithizone in small portions to impart a yellow tint to the soln. Shake to extract the colored Zn salt. Allow to separate, rinse delivery tube with a few drops of the solvent layer, and draw off remainder thru a dry ashless filter (to remove traces of moisture) into a weighing bottle and stopper to prevent evaporation.

Compare the color in a Duboscq colorimeter, using micro cups and a green color filter, against 5 ml of the dilute Zn soln treated in exactly the same manner.

$$\% \text{ Zn} = \frac{sRF}{R_1}$$

( $F = 250$  for 0.40 g aliquot);

$$= \frac{0.0025R}{R_1};$$

$$\text{p.p.m. Zn} = \frac{25R}{R_1}, \text{ where}$$

$s$  = g of the standard used;

$R$  = scale reading at which standard was set;

$R_1$  = scale reading of unknown; and

$F$  = factor for converting the aliquot to percentage or p.p.m.

Since a small quantity of Zn (ca 2 p.p.m.) will usually be found in the blank after careful purification of the reagents,  $\frac{(s+B)R}{R_1}$  should be substituted in the calculation and the blank deducted.

48

MANGANESE—TENTATIVE.—See XII, 13, or XXVI, 16 and 17.

#### SELENIUM<sup>11</sup>—TENTATIVE

49

#### PREPARATION OF SAMPLE

Place 5–10 g (dry weight) of sample in 600 ml Pyrex beaker or Kjeldahl flask and add 0.5 g of HgO, and a cooled mixture of 50 ml of H<sub>2</sub>SO<sub>4</sub> and volume of HNO<sub>3</sub> equal to 10 ml per g of sample taken. Mix thoroly and allow to stand 30 min. Heat *gently* until NO<sub>2</sub> fumes are no longer evolved and soln turns to dark brown or SO<sub>3</sub> fumes appear. (The Hg can best be added in soln in HNO<sub>3</sub>.)

50

#### ISOLATION

Add 25 ml of H<sub>2</sub>O to the cold H<sub>2</sub>SO<sub>4</sub> digest, cool again, and transfer mixture to a 500 ml flask, which is a part of an all-glass distilling apparatus consisting of a round-bottomed flask, still head (with standard taper joints connecting thermometer, condenser, and flask), and adapter. Have adapter dip into a soln consisting of 5–10 ml of H<sub>2</sub>O and 2–5 ml of HBr + 0.5% Br<sub>2</sub>. Complete the transfer with 50–60 ml of HBr containing 0.5% by volume of free Br<sub>2</sub>. Distil until temp. reaches 130°, keeping receiving flask cool. (The temp. of constant boiling HBr is 126°. At 130° all selenium and practically all the HBr is distilled.) If distillate contains insoluble material, filter thru asbestos and wash with 5–10 ml of cold H<sub>2</sub>O. Saturate filtrate with SO<sub>2</sub> gas, add 0.1 g of NH<sub>2</sub>OH.HCl, and warm on steam bath to 80° for 15 min. Allow mixture to cool, filter thru asbestos Gooch or Jena glass filter No. 4, and wash with 5–10 ml of cold H<sub>2</sub>O. (The filtrate may be saved for recovery of HBr.)

51

#### VOLUMETRIC DETERMINATION WITH STARCH INDICATOR

Estimate amount of precipitated Se on filter (used in determining quantity of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln to be added later in titration). Dissolve the Se in 1–2 ml of 48% HBr containing 1% by volume of Br<sub>2</sub>, using a few drops to rinse precipitation flask. Wash with minimum quantity of H<sub>2</sub>O so as to keep volume of filtrate below 20 ml at most and at ca 10 ml for amounts of Se of 20 micrograms and under. Transfer filtrate and washings to 30 or 50 ml beaker. Prepare a few standards containing amounts of Se in general range of samples, and 2–3 blanks. Dilute standards and blanks to about volume of samples and add same volume of HBr + Br<sub>2</sub> as in samples.

To samples, standards, and blanks, add strong soln of  $\text{H}_2\text{SO}_3$  until  $\text{Br}_2$  color nearly disappears. (In case all  $\text{Br}$  is reduced, add  $\text{HBr} + \text{Br}_2$ , dropwise, until color reappears.) Decolorize with 1–2 drops of 5% aqueous phenol. (It is desirable to reduce color to light yellow before adding phenol since tribromophenol is precipitated with excess  $\text{Br}$ . The presence of the precipitate, while undesirable, does not ruin the determination.)

Use stirrer and 10 ml buret provided with an extension to dip into soln being titrated, with tip so constricted as to make possible addition of soln in 0.01–0.02 ml portions. Place soln being titrated on white surface with white background and view by reflected light.

To the decolorized soln in the 30–50 ml beaker add ca 1 ml of freshly prepared starch soln. Then add rapidly from buret moderate excess of 0.01, 0.001, or 0.0005  $N$   $\text{Na}_2\text{S}_2\text{O}_3$ , using estimate of precipitated  $\text{Se}$  as guide. (1 ml of 0.01  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  is roughly equivalent to 200 micrograms of  $\text{Se}$ ; 1 ml of 0.001  $N$  to 20 micrograms; 1 ml of 0.0005  $N$  to 10 micrograms.)

Add ca 2 ml more than the estimated equivalent of  $\text{Na}_2\text{S}_2\text{O}_3$  and so select normality as to keep volume added between 2 and 10 ml. After ca 20–30 seconds add rapidly from buret a soln of  $\text{I}_2$  (approximately same strength as  $\text{Na}_2\text{S}_2\text{O}_3$  used) until permanent blue color appears. If less than 1 ml of  $\text{I}_2$  has been added, add 2 ml more of  $\text{Na}_2\text{S}_2\text{O}_3$  and then  $\text{I}_2$  until at least 1 ml is needed to give blue color. Then add slowly from dipping buret  $\text{Na}_2\text{S}_2\text{O}_3$  of same strength as before until color is same as a blank containing  $\text{H}_2\text{O}$  and 1 ml of starch soln.

## 52

## CALCULATIONS

Add total volumes of  $\text{I}_2$  and of  $\text{Na}_2\text{S}_2\text{O}_3$  for each determination.

*Blanks.*—Divide volume of  $\text{Na}_2\text{S}_2\text{O}_3$  by volume of  $\text{I}_2$  to get factor for conversion of volumes of  $\text{I}_2$  to the equivalent volumes of  $\text{Na}_2\text{S}_2\text{O}_3$ . Average the results.

*Standards.*—Multiply volumes of  $\text{I}_2$  by the  $\text{I}_2$ - $\text{Na}_2\text{S}_2\text{O}_3$  conversion factor, and subtract product from total volume of  $\text{Na}_2\text{S}_2\text{O}_3$ . Divide this number into quantity of  $\text{Se}$  in standard to get the micrograms  $\text{Se}/\text{ml}$   $\text{Na}_2\text{S}_2\text{O}_3$ . Average the results.

*Samples.*—Calculate volume of  $\text{Na}_2\text{S}_2\text{O}_3$  used in reduction of the  $\text{Se}$  as directed under "Standards" and multiply by micrograms  $\text{Se}/\text{ml}$   $\text{Na}_2\text{S}_2\text{O}_3$  value to get total quantity of  $\text{Se}$  in sample in micrograms. Divide total quantity by weight of sample in grams taken to get the p.p.m. of  $\text{Se}$ .

## SELECTED REFERENCES

- <sup>1</sup> U. S. Dept. Agr. Bur. Chem. Circ., 102 (1912); J. Assoc. Official Agr. Chem., 7, 48 (1923); 16, 398 (1933); 18, 189, 506 (1935); 19, 95 (1936).
- <sup>2</sup> J. Assoc. Official Agr. Chem., 16, 75 (1933); 17, 70, 202 (1934); 19, 95 (1936).
- <sup>3</sup> Ibid., 17, 108 (1934); 18, 315 (1935); 19, 130 (1936).
- <sup>4</sup> Ibid., 20, 622 (1937).
- <sup>5</sup> Ind. Eng. Chem., Anal. Ed., 7, 285 (1935).
- <sup>6</sup> J. Assoc. Official Agr. Chem., 18, 638 (1935); 22, 341 (1939).
- <sup>7</sup> Ibid., 1, 257 (1915).
- <sup>8</sup> Original Communications, VIII Intern. Cong. Appl. Chem., 18, 35 (1912).
- <sup>9</sup> J. Assoc. Official Agr. Chem., 13, 426 (1930).
- <sup>10</sup> Ibid., 22, 333 (1939).
- <sup>11</sup> Ibid., 316, 346.

## XXX. NUTS AND NUT PRODUCTS<sup>1</sup>

1

### PREPARATION OF SAMPLE—TENTATIVE

Without delay, transfer all samples received in packages to glass-stoppered containers or Mason jars and keep in a cool, dark place. Prepare various samples for analysis as follows:

(a) *Fresh shelled nuts*.—Cut nuts into small pieces and weigh sample desired for analysis. Transfer weighed sample to mortar and grind to fine state of division with pestle. In transferring ground material from mortar to required flask, use a portion of the solvent to clean out mortar.

(b) *Shredded or prepared coconut*.—Transfer weighed sample to mortar and proceed as directed under (a).

(c) *Almond paste, kernel paste, peanut butter, etc.*—Transfer sample to Mason jar or beaker ca three times the size of sample and mix carefully with a stiff-bladed spatula or knife. *Almond paste and similar products containing added H<sub>2</sub>O must be freed of moisture before analysis.*

### MOISTURE

2

*Method I.—Tentative.—See XXVII, 3.*

3

*Method II.—Tentative*

Weigh 2 g of the product into flat-bottomed dish. If necessary to secure a thin layer of the material, add a few ml of H<sub>2</sub>O, and mix thoroly. Dry at 70° under pressure not to exceed 100 mm of Hg until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg.

4

ASH—TENTATIVE.—See XXXIV, 9 or 10.

5

### CRUDE FIBER—TENTATIVE

Use the fat-free material and proceed as directed under XXVII, 27.

### FAT, CONSTANTS OF FAT, AND PROTEIN

6

*Method I.—Tentative*

Weigh into 200 ml volumetric flask 2–3 g of material. Add 100 ml of CHCl<sub>3</sub> from pipet, washing down sample with stream of CHCl<sub>3</sub>. Stopper flask and shake frequently during 30 min. Filter soln thru 11 cm fluted paper, and as soon as 25 ml of soln has been filtered, pipet out two 10 ml portions, using same pipet. Transfer one aliquot to weighed crystallizing dish, 50×35 mm, and evaporate solvent on steam bath. Dry dish and contents at 100° for 30 min., cool, and weigh. Use weight obtained for calculating percentage of fat and I number of the fat. Determine refractive index of the dried residue. Transfer the other 10 ml portion to glass-stoppered flask or bottle, add 30 ml of Hanus soln, XXXI, 18(a), and proceed as directed under XXXI, 19. Complete filtration of the CHCl<sub>3</sub> extract. Transfer extracted residue to Kjeldahl flask, wash the volumetric flask thoroly with boiling H<sub>2</sub>O, and transfer washings to digestion flask. Determine N as directed under II, 23.  $N \times 6.25$  = protein.

7

*Method II.—Tentative*

Proceed as directed under XIX, 12, using 300 ml Erlenmeyer receiving flask in place of the 150 ml flask. (A fritted Jena glass Büchner filter is more convenient

than the Knorr extraction tube.) To facilitate filtration, mix equal volume of filter cel with peanut butter. Determine constants of fat and protein as directed under 6.

#### SUGAR AND SALT

8

##### *Method I.—Tentative*

Extract ca 10 g of sample in 8 oz nursing bottle with two 100 ml portions of petroleum benzin, in each case shaking for 5 min., centrifuging, and pouring off supernatant liquid. Warm bottle to drive off remaining solvent and transfer dry residue to 100–150 ml separatory funnel. Complete transfer with mixture of 3 volumes of  $\text{CCl}_4$  and 1 volume of  $\text{CHCl}_3$ . Add more of the liquid and shake mixture vigorously. Wash down sides of funnel, using total quantity of 60–80 ml of the liquid. Stopper funnel and allow to stand overnight. Remove sugar and salt that have settled out by opening stopcock quickly, and if necessary pull out stopcock. Evaporate off liquid on steam bath or other warm place and dissolve residue in hot  $\text{H}_2\text{O}$ . Transfer soln to 100 ml volumetric flask with hot  $\text{H}_2\text{O}$ , cool, make up to mark, and mix. Filter thru small, dry filter paper. Determine chlorides in 20 ml aliquot by titration with  $\text{AgNO}_3$ , using dichromate indicator. Determine reducing sugars before and after inversion as directed under XXXIV, 38.

9

##### *Method II.—Tentative*

Weigh 10 g of the material on filter (with peanut butter thoroly mix 5 g of filter cel with weighed charge) and extract with suction 10 successive times at 3 min. intervals with 50 ml of petroleum benzin (b.p. below  $60^\circ$ ). (A fritted Jena glass Büchner filter is most convenient.) At beginning of each extraction stir soln well with glass rod flattened at end. After defatting, macerate well in porcelain mortar and transfer material with hot  $\text{H}_2\text{O}$  to 250 ml Pyrex or similar volumetric flask. If frothing occurs, add a few drops of caprylic alcohol, breaking up foam with glass rod. Pass hot  $\text{H}_2\text{O}$  thru filter and add to  $\text{H}_2\text{O}$  in flask until total volume is ca 200 ml. Digest in vessel of boiling  $\text{H}_2\text{O}$  for 15 min., cool under tap, and add ca 5 ml of a saturated neutral Pb acetate soln. Make to mark at room temp., shake well, transfer to centrifuge bottle, and whirl at 2000 r.p.m. for 15 min. Filter on  $18\frac{1}{2}$  cm folded filter, rejecting first 25 ml of filtrate. De-lead with K oxalate and again filter, rejecting first 25 ml of filtrate. Determine reducing sugars before and after inversion and multiply by 0.95 to obtain the sucrose. Multiply results obtained by 0.97 to correct for volume of insoluble material. In the case of peanut butter, multiply by factor 0.95 to correct for volume occupied by filter cel and peanut butter. Determine chlorides as directed under 8.

10

#### DEXTROSE OR D-GLUCOSE—TENTATIVE

Proceed as directed under 8 or 9 and XXXIV, 38.

#### PEANUT BUTTER

11

##### PRELIMINARY PROCEDURE—TENTATIVE

Make microscopical examination to detect addition of starch or any off-grade material not identifiable chemically.

12

##### STARCH—TENTATIVE

Weigh 4–5 g of sample by difference into 8 oz nursing bottle and extract twice with 50 ml portions of petroleum benzin, in each case shaking for 5 min. Wash down sides of bottle with petroleum benzin, centrifuge, and pour off solvent, disregarding

opalescence. Warm bottle to drive off remaining solvent, and transfer residue to mortar and grind. Return the fine powder to bottle with aid of 100 ml of 10% NaCl soln. Shake bottle for 15 min., wash down sides with salt soln, centrifuge well, and pour off supernatant liquid, disregarding opalescence. Repeat this procedure twice. Extract once in same manner with 70% alcohol and then once with H<sub>2</sub>O, shaking for 1–2 min. in each case. Drain bottle for several minutes, chill, and add from pipet 100 ml of HCl soln (20.5–21.0 g of HCl per 100 ml) at temp. not higher than 15°. Shake vigorously for 3 min., centrifuge well, and pour off soln thru pledget of cotton in stem of funnel. Cool soln to temp. at which the HCl was added, and pipet off 50 ml into nursing bottle containing 115 ml of 95% alcohol. Shake with whirling motion for 1 min., let stand for 2 min., centrifuge for 2 min., pour off thru a weighed Gooch crucible containing a thin pad of asbestos, and add 50 ml of 70% alcohol to precipitate. Stopper bottle, shake vigorously, wash down sides with the 70% alcohol, centrifuge lightly, and pour off thru crucible. Repeat once with 70% alcohol and once with 95% alcohol. Dry crucible and contents for 1.5 hours at 130° in air, or for 5 hours at 98–100° in vacuo. Cover crucible, place in desiccator containing efficient desiccant, and weigh crucible as soon as it has attained room temp.

### ALMOND PASTE, KERNEL PASTE, ETC.

13

#### SEPARATION AND PREPARATION OF THE OIL—TENTATIVE

Dry the paste in oven and extract repeatedly with petroleum benzin by rubbing in mortar and pouring off solvent thru filter. Evaporate benzin on steam bath and test extracted oil.

14

#### *Bieber's Test<sup>2</sup>*

Agitate 5 volumes of oil with 1 volume of mixture of equal parts, by weight, of H<sub>2</sub>SO<sub>4</sub>, fuming HNO<sub>3</sub>, and H<sub>2</sub>O. Pure almond oil does not change color; after standing for some time apricot kernel oil gives pink peach-blossom color, and peach kernel oil, faint pink coloration. It is advisable to prepare reagent fresh for each set of tests. It is doubtful whether less than 25% of apricot kernel oil can be detected.

15

#### *Nitric Acid Test<sup>3</sup>*

On being shaken with HNO<sub>3</sub>, almond oil remains colorless or becomes slightly yellow; apricot kernel oil assumes a color ranging from orange-yellow to red; and peach kernel oil becomes yellowish brown.

16

#### *Kreis Test<sup>4</sup>*

Mix 1 volume of the oil in test tube with 1 volume of 0.10% soln of phloroglucinol in ether, and pour 1 volume of HNO<sub>3</sub> down side of tube. Keep tube cold. A red ring forms at junction of two liquids when apricot kernel, sesame, or cottonseed oil is present. Almond oil gives no red color—or, at most, only a light pink.

The presence or absence of other oils (such as cottonseed, sesame, peanut, or olive) may be detected by the variation in constants and by characteristic tests. It is seldom that these oils are found unless added starch is present.

17

#### MICROSCOPIC EXAMINATION

In connection with the microscopic examination of almond paste and other products containing ground almonds, attention is called to the following publications, which give detailed descriptions and illustrations of the tissue elements:

Young, W. J.—A Study of Nuts with Special Reference to Microscopic Identification, U. S. Dept. Agr. Bur. Chem. Bull. 160 (1912).



Hamig, E.—Z. Nahr. Genussm., **21**, 577 (1911).

Pease, V. A.—Notes on the Histology of the Almond, J. Agr. Research, **41**, 789–800 (1930).

Winton, Andrew L. and Kate B.—The Structure and Composition of Foods, Vol. 1, p. 476 (1932).

### SHREDDED COCONUT

18

#### GLYCEROL—TENTATIVE

Extract with suction 4 times, 4 g of the shredded coconut (dried in vacuo at 70° for 5 or 6 hours) on filter (fritted Jena glass Büchner filter is most convenient), using for each extraction 50 ml of petroleum benzin (b.p. below 65°), and allowing 3 min. intervals between extractions. Use flattened glass rod for stirring. After removing fat, extract residue on filter with four 50 ml portions of absolute alcohol, allowing 3 min. intervals with stirring, as before. Make the absolute alcohol extract to 250 ml with absolute alcohol at room temp. Pipet 100 ml into 500 ml Erlenmeyer flask, and add 5 ml of H<sub>2</sub>O and a paste made by adding hot H<sub>2</sub>O to 2 or 3 g of Ba(OH)<sub>2</sub> in small mortar. Heat mixture on steam bath to boiling and boil ca 1 min.; transfer to 250 ml centrifuge bottle, and centrifuge at 2000 r.p.m. ca 5 min. Transfer the clear liquid to large porcelain dish and wash residue in centrifuge bottle with 50–75 ml of absolute alcohol, stirring with glass rod and centrifuging as before. Evaporate on steam bath at temp. below 70° to a few drops, or almost dryness. Transfer to 50 ml glass-stoppered cylinder with 10 ml of absolute alcohol and wash dish with two 5 ml portions of absolute alcohol. Further wash dish with three 10 ml portions of anhydrous ether, shaking glass-stoppered cylinder thoroly after each addition of the anhydrous ether. Transfer to sediment tube and centrifuge for 10 min. at a speed of 3200 r.p.m. Transfer clear soln in sediment tube to evaporating dish, preferably Pt, and wash sediment tube with 25 ml of mixture of absolute alcohol and anhydrous ether (2:3), stirring with glass stirring rod and centrifuging as before. Evaporate on steam bath at temp. of 85–90° to ca 5 ml, add 20 ml of H<sub>2</sub>O, and evaporate to ca 5 ml; repeat this operation twice. Transfer residue with hot H<sub>2</sub>O to 50 ml volumetric flask and proceed as directed under XXXIII, 74.

### SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., **18**, 419 (1935).

<sup>2</sup> Z. Anal. Chem., **17**, 264 (1878); Pharm. Centralb., **18**, 315.

<sup>3</sup> Schweizer Lebensmittelbuch, 3rd ed., p. 43 (1917).

<sup>4</sup> Chem. Ztg., **26**, 897 (1902).

## XXXI. OILS, FATS, AND WAXES

1

### PREPARATION OF SAMPLE—OFFICIAL

Melt solid fats and filter by means of hot water funnel or similar apparatus. Make the different determinations on samples of this melted, homogeneous mass. Filter oils that are not clear. Keep oils and fats in cool place and protected from light and air, otherwise they will soon become rancid. Weigh out at one time as many portions as are needed for the various determinations, using small beaker or weighing buret.

### MOISTURE AND VOLATILE MATTER<sup>1</sup>

2

#### *Vacuum Oven Method—Official*

Soften sample if necessary by means of gentle heat, taking care not to melt it. When sufficiently softened, mix thoroly with mechanical egg beater or other equally effective mechanical mixer.

Weigh 5 g ( $\pm 0.2$  g) of the prepared sample into shallow glass moisture dish ca 6–7 cm in diameter and 4 cm deep. Dry to constant weight in vacuum oven (F.A.C. standard or equivalent) at uniform temp. not less than 20° nor more than 25° above boiling point of H<sub>2</sub>O at working pressure, which should not exceed 100 mm of Hg. Constant weight is attained when successive dryings for 1 hour periods show additional loss of not more than 0.05%. Cool sample in efficient desiccator (30 min.) and reweigh. Report percentage loss in weight as moisture and volatile matter.

### SPECIFIC GRAVITY (APPARENT)

#### *At 25/25°—Official*

3

### STANDARDIZATION OF PYCNOMETER

Carefully clean pycnometer by filling it with saturated soln of CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub> and allowing to stand for several hours. Empty pycnometer and rinse thoroly with H<sub>2</sub>O; fill it with recently boiled H<sub>2</sub>O previously cooled to ca 20° and place in constant temp. bath at 25°. At end of 30 min. adjust level of the H<sub>2</sub>O to proper point on pycnometer and put the perforated cap or stopper in place; remove from bath, wipe dry with clean cloth or towel, allow to stand for 30 min., and weigh. Empty pycnometer, rinse several times with alcohol and then with ether, allow it to become perfectly dry, remove ether vapor, and weigh. Ascertain weight of contained H<sub>2</sub>O at 25° by subtracting weight of pycnometer from its weight when full.

4

### DETERMINATION

Fill the clean, dry pycnometer with the oil previously cooled to ca 20°, place in constant temp. bath at 25° for 30 min., adjust level of oil to proper point on pycnometer, and put the cap or stopper in place; remove from bath, wipe dry, and weigh as directed under 3. Subtract weight of empty pycnometer from its weight when filled with oil and divide difference by weight of H<sub>2</sub>O at 25°, as determined under 3. The quotient is the sp. gr. at 25/25° (apparent).

5

### TEMPERATURE CORRECTION FOR SPECIFIC GRAVITY OF OILS—OFFICIAL

If the sp. gr. of the oil is determined at other than standard temp., the approximate sp. gr. at 25° may be calculated by means of following formula:

$$G = G' + 0.0007 (T' - 25^\circ), \text{ in which} \\ G = \text{sp. gr. at } 25^\circ;$$

$$G' = \text{sp. gr. at } \frac{T}{25^{\circ}};$$

$T$  = temp. at which the sp. gr. was determined; and  
 0.0007 = mean correction<sup>2</sup> for  $1^{\circ}$ .

*At the Temperature of Boiling Water—Official*

6

## STANDARDIZATION OF FLASKS

(a) Weigh a 25–30 ml sp. gr. flask and fill with freshly boiled hot  $\text{H}_2\text{O}$ . Place in briskly boiling water bath for 30 min., replacing any evaporation from flask by addition of boiling  $\text{H}_2\text{O}$ . Insert stopper, previously heated to  $100^{\circ}$ , remove flask, cool, and weigh.

(b) The following formula may be used for calculating the weight of  $\text{H}_2\text{O}$  ( $W^T$ ) that a given flask will hold at  $T^{\circ}$  (weighed in air with brass weights at temp. of room) from weight of  $\text{H}_2\text{O}$  ( $W^t$ ) (weighed in air with brass weights at temp. of room) contained therein at  $t^{\circ}$ :

$$W^T = W^t \frac{d^T}{d^t} [1 + 0.000026 (T - t)], \text{ in which}$$

$d^T$  = density of  $\text{H}_2\text{O}$  at  $T^{\circ}$ ; and

$d^t$  = density of  $\text{H}_2\text{O}$  at  $t^{\circ}$ .

7

## DETERMINATION

Fill the dry flask with the dry, hot, freshly filtered fat, which should be entirely free from air bubbles, and keep in water bath for 30 min. at temp. of boiling  $\text{H}_2\text{O}$ . Insert stopper, previously heated to  $100^{\circ}$ , cool, and weigh. Divide weight of contained fat by weight of contained  $\text{H}_2\text{O}$  previously found to obtain the sp. gr.

The weight of  $\text{H}_2\text{O}$  at boiling temp. must be determined under the barometric conditions prevailing at time the determination is made.

## INDEX OF REFRACTION

8

## GENERAL DIRECTIONS—OFFICIAL

Place instrument in such position that diffused daylight or some form of artificial light can readily be obtained for illumination. Circulate thru prisms a stream of  $\text{H}_2\text{O}$  of constant temp. Determine index of refraction with any standard instrument, reading oils at  $20^{\circ}$  and fats at  $40^{\circ}$ . The readings of the Zeiss butyro-refractometer on fats may be reduced to standard temp. by following formula:<sup>3</sup>

$$R = R' + 0.55 (T' - T), \text{ in which}$$

$R$  = reading reduced to temp.,  $T$ ;

$R'$  = reading at  $T'$ ;

$T'$  = temp. at which reading  $R'$  is made;

$T$  = standard temp.; and

0.55 = correction in scale divisions for  $1^{\circ}$ .

With oils the factor 0.58 is substituted in the formula for 0.55, because they have a higher index of refraction. The readings of instruments that give the index of refraction directly can be reduced to standard temp. by substituting the factor 0.00038 for 0.55 in the formula. As the temp. rises the refractive index falls. The instrument used may be standardized with  $\text{H}_2\text{O}$  at  $20^{\circ}$ , the theoretical refractive index of  $\text{H}_2\text{O}$  at that temp. being 1.3330. Any correction found should be made on all readings. The index of refraction varies with the sp. gr. and in the same direction. If the

results appear abnormal, compare the specific refractive power<sup>4</sup> with the normal. Calculate the specific refractive power from the formula  $\frac{N-1}{D}$ , in which  $N$  equals the refractive index and  $D$  the sp. gr. According to Proctor,<sup>5</sup> the Lorenz formula,  $\frac{N^2-1}{(N^2+2)D}$ , gives much more satisfactory results than  $\frac{N-1}{D}$ .

9

*I. By Means of the Abbé Refractometer—Official*

To charge the instrument, open the double prism by means of the screw head and place a few drops of the sample on the prism or, if preferred, open prisms slightly by turning screw head and pour a few drops of sample into the funnel-shaped aperture between prisms. Close prisms firmly by tightening screw head. Allow instrument to stand for a few minutes before reading is made, so that temp. of sample and instrument will be the same.

The method of measurement is based upon the observation of the position of the *border line of total reflection* in relation to the faces of a prism of flint glass. Bring this border line into field of vision of telescope by rotating the double prism by means of the alidade in following manner: Hold the sector firmly and move the alidade backward or forward until field of vision is divided into light and dark portion. The line dividing these portions is the "border line," and, as a rule, will not be a sharp line but a band of color. The colors are eliminated by rotating screw head of compensator until a sharp, colorless line is obtained. Adjust the border line so that it falls on the point of intersection of the cross hairs. Read the refractive index of the substance directly on the scale of the sector. Check correctness of instrument as directed under 8, or by means of the quartz plate that accompanies it, using monobromonaphthalene, and make the necessary correction in reading.

10

*Butyro-refractometer readings and indices of refraction*

READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION
40.0	1.4524	50.0	1.4593	60.0	1.4659	70.0	1.4723
40.5	1.4527	50.5	1.4596	60.5	1.4662	70.5	1.4726
41.0	1.4531	51.0	1.4600	61.0	1.4665	71.0	1.4729
41.5	1.4534	51.5	1.4603	61.5	1.4668	71.5	1.4732
42.0	1.4538	52.0	1.4607	62.0	1.4672	72.0	1.4735
42.5	1.4541	52.5	1.4610	62.5	1.4675	72.5	1.4738
43.0	1.4545	53.0	1.4613	63.0	1.4678	73.0	1.4741
43.5	1.4548	53.5	1.4616	63.5	1.4681	73.5	1.4744
44.0	1.4552	54.0	1.4619	64.0	1.4685	74.0	1.4747
44.5	1.4555	54.5	1.4623	64.5	1.4688	74.5	1.4750
45.0	1.4558	55.0	1.4626	65.0	1.4691	75.0	1.4753
45.5	1.4562	55.5	1.4629	65.5	1.4694	75.5	1.4756
46.0	1.4565	56.0	1.4633	66.0	1.4697	76.0	1.4759
46.5	1.4569	56.5	1.4636	66.5	1.4700	76.5	1.4762
47.0	1.4572	57.0	1.4639	67.0	1.4704	77.0	1.4765
47.5	1.4576	57.5	1.4642	67.5	1.4707	77.5	1.4768
48.0	1.4579	58.0	1.4646	68.0	1.4710	78.0	1.4771
48.5	1.4583	58.5	1.4649	68.5	1.4713	78.5	1.4774
49.0	1.4586	59.0	1.4652	69.0	1.4717	79.0	1.4777
49.5	1.4590	59.5	1.4656	69.5	1.4720	79.5	1.4780

11

*II. By Means of the Zeiss Butyro-Refractometer—Official*

Place 2 or 3 drops of the filtered fat on surface of lower prism. Close prisms and adjust mirror until it gives sharpest reading. If reading is indistinct after running  $H_2O$  of a constant temp. thru instrument for some time, the fat is unevenly distributed on surfaces of prism. As index of refraction is greatly affected by temp., use care to keep latter constant. Carefully adjust instrument by means of the standard fluid that is supplied with it. Convert degrees of instrument into refractive indices from the table under 10.

## MELTING POINT OF FATS AND FATTY ACIDS

*Wiley Method—Official*

12

## REAGENT

*Alcohol-water mixture.*—The sp. gr. should be the same as that of fat to be examined. Prepare by boiling, separately,  $H_2O$  and alcohol for 10 min. to remove gases that may be held in soln. While still hot pour the  $H_2O$  into test tube until it is almost half full. Nearly fill test tube with the hot alcohol, pouring it down side of inclined tube to avoid too much mixing. If the alcohol is added after the  $H_2O$  has cooled, air bubbles will make mixture unfit for use.

13

## DETERMINATION

Allow the melted and filtered fat to fall a distance of 15–20 cm from dropping tube upon piece of ice or upon surface of cold Hg. The disks thus formed should be 1–1.5 cm in diameter and should weigh ca 200 mg. Remove disks when solid, and allow to stand 2–3 hours in order to obtain normal melting point.

Place a 30 × 3.5 cm test tube, containing the alcohol-water mixture, in tall 35 × 10 cm beaker containing ice and  $H_2O$ , and leave until mixture is cold. Drop disk of fat into tube. It will sink immediately to a point where density of the alcohol-water mixture is exactly equivalent to its own. Lower accurate thermometer, which can be read to 0.1°, into test tube until bulb is just above disk. In order to secure even temp. in all parts of the alcohol-water mixture around disk, stir gently with thermometer. Slowly heat the  $H_2O$  in beaker, constantly stirring it by means of air blast or other suitable device.

When temp. of the alcohol-water mixture rises to ca 6° below melting point of the fat, the disk of fat begins to shrivel and gradually rolls up into an irregular mass. Lower thermometer until fat particle is even with center of bulb. Rotate thermometer bulb gently and so regulate heat that ca 10 min. is required for last 2° increase in temp. As soon as fat mass becomes spherical, read thermometer. Remove tube from bath and again cool. Place in bath a second tube containing the alcohol-water mixture. The test tube is of sufficiently low temp. to cool bath to desired point. After first or preliminary determination, regulate temp. of bath so as to obtain a maximum of ca 1.5° above melting point of fat under examination.

If edge of disk touches sides of tube, make a new determination. Run triplicate determinations. The second and third results should agree closely.

14

*Capillary Tube Method<sup>a</sup>—Official*

Draw the melted fat or fatty acids into thin-walled capillary tube. Use column of fat 1–2 cm long, according to length of thermometer bulb. Seal one end of tube and cool on ice 12–15 hours. Attach capillary tube to bulb of accurate thermometer graduated to 0.2°, immerse in large test tube of  $H_2O$  surrounded by beaker of  $H_2O$ ,

and heat very slowly. An apparatus similar to that indicated in Fig. 40 may be used. Take as melting point the temp. at which the substance becomes transparent.

#### TITER TEST

##### *Alcoholic or Aqueous Sodium Hydroxide Method—Official*

15

##### SPECIFICATIONS FOR TITER TEST THERMOMETERS<sup>7</sup>—OFFICIAL

The original specification for the titer test thermometer is about 20 years old and on account of certain arbitrary limits in specifications this thermometer has always been difficult and expensive to manufacture. It appears, furthermore, that it was originally designed to be read to 1/10 or 1/5 of a division, that is, to 0.01° or 0.02°, whereas, in practice, it is read to nearest division, or perhaps occasionally to 1/2 division. The original specifications were difficult principally because they were designed to keep the thermometer as short as possible, and this resulted in crowding the division marks so close together that reading was not easy. A slightly shorter, much more easily readable thermometer is obtained by subdividing the scale to 0.2°C. with a scale sufficiently open to make reading to 1/2 division, 0.1°, easy. This thermometer has been designed so as to cause no undue difficulties in manufacture, and at the same time to meet fully the requirements for accuracy in the titer test.

*Type.*—Etched stem, glass.

*Liquid.*—Mercury.

*Range and subdivision.*—Minus 2 to +66° in 0.2°, with expansion chamber at top.

*Total length.*—370–380 mm.

*Stem.*—Plain front, enamel back, suitable thermometer tubing. Diameter, 6–7 mm.

*Bulb.*—Corning normal or equally suitable thermometric glass. Diameter not less than 5.5 mm but not greater than that of the stem. Length, 20–30 mm.

*Distance to –2° mark from bottom of bulb.*—45–60 mm.

*Distance to 66° mark from top of thermometer.*—20–50 mm.

*Length of unchanged capillary.*—Between top of bulb and the first graduation mark, 13 mm, and between the last graduation mark and the expansion chamber at the top, 10 mm.

*Top finish.*—Glass ring or knob.

*Filling above mercury.*—Nitrogen or other suitable gas, or vacuum.

*Graduation.*—All lines, figures, and letters to be clear cut and distinct. Each degree mark to be longer than the remaining lines. Graduations to be numbered at each multiple of 2°.

*Immersion.*—Total.

*Special marking. A.O.A.C. titer test.*—A serial number, and the manufacturer's name or trademark shall be etched upon the stem. The marking "0.2°C." shall be marked on the front of the stem above the scale.

*Scale error.*—The error at any point on the scale, when the thermometer is standardized at total immersion, shall not exceed 0.2°C.

*Case.*—The thermometer shall be supplied in a suitable case on which shall appear the marking: *A.O.A.C. Titer Test, –2° to 66°C. in 0.2°.*

**NOTE:** For the purpose of interpreting these specifications the following definitions apply:

The total length is the over-all length of the finished instrument.

The diameter is that measured with a ring gage.

The length of the bulb is the distance from the bottom of the bulb to the beginning of the enamel backing.

The top of the thermometer is the top of the finished instrument.

Saponify 75 g of the sample in metal dish with 60 ml of 30% NaOH soln (36° Baumé) and 75 ml of alcohol or 120 ml of  $H_2O$ . Evaporate to dryness over very low flame or on iron or asbestos plate, stirring constantly. Dissolve the dry soap in 1 liter of boiling  $H_2O$ , and if alcohol has been used boil for 40 min. to remove

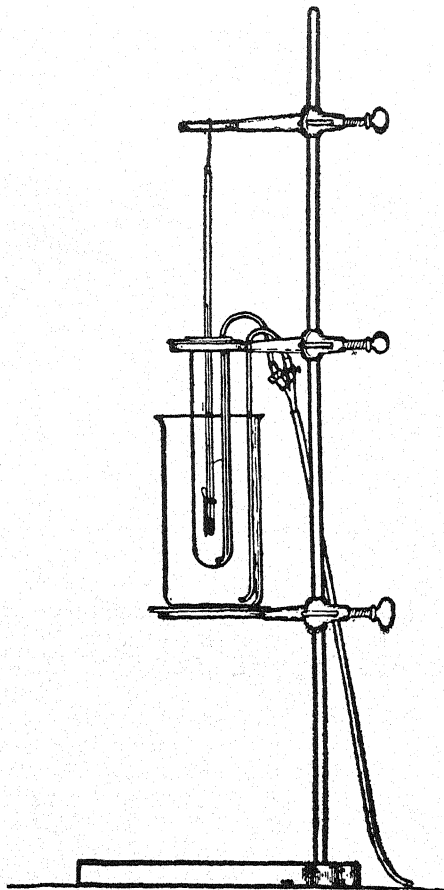


FIG. 40.—APPARATUS FOR DETERMINATION OF MELTING POINT

it, adding sufficient  $H_2O$  to replace that lost in boiling. Liberate fatty acids by adding 100 ml of  $H_2SO_4$  (1+3) and boil until they form a clear, transparent layer. Wash fatty acids with boiling  $H_2O$  until free from  $H_2SO_4$ , collect in small beaker, and place on steam bath until the  $H_2O$  has settled and fatty acids are clear. Decant into dry beaker, filter while hot, and dry 20 min. at 100°. When dried, cool fatty acids to 15–20° above expected titer and transfer to titer tube, 25 by 100 mm (1×4") and made of glass ca 1 mm in thickness. Place in 16 oz wide-mouthed bottle of clear glass, 70 by 150 mm (2.8×6"), fitted with perforated cork so as to hold tube rigidly when in position. Suspend the standard thermometer so that it can be used as a stirrer and stir mass slowly until the Hg remains stationary for 30 seconds. Allow thermometer to hang quietly, with bulb in center of mass, and observe rise of Hg column. The highest point to which it rises is regarded as the titer of the fatty acids.

Test fatty acids for complete saponification as follows: Place 3 ml in test tube and add 15 ml of alcohol. Bring mixture to boil and add equal volume of  $NH_4OH$  (1+2). A clear soln should result. Make titer at ca 20° for all fats having titer above 30°, and at 10° below titer for all other fats.

*Glycerol-Potassium Hydroxide Method—Official*

Heat 75 ml of glycerol KOH soln (25 g of KOH in 100 ml of U.S.P. glycerol) to 150° in an 800 ml beaker and add 50 ml of the oil or melted fat, previously filtered if necessary to remove foreign substances. Saponification often takes place almost immediately, but heating and frequent stirring should be continued for 15 min. Do not use temp. much above 150°. When saponification is complete, as indicated by the perfectly homogeneous soln, pour soap into 800 ml casserole containing ca 500 ml of nearly boiling  $H_2O$ ; add carefully 50 ml of  $H_2SO_4$  (1+3); and heat soln,

with frequent stirring, until layer of fatty acids separates out perfectly clear. Transfer fatty acids to tall separatory funnel, wash 3 or 4 times with boiling  $H_2O$  to remove all mineral acids, draw fatty acids off into small beaker, and allow to stand on steam bath until the  $H_2O$  has settled out and acids are clear. Filter into dry beaker and heat to  $150^\circ$  on thin asbestos plate, stirring continually with thermometer; transfer to titer tube, fill it to within 2.5 cm of top, and take titer as directed under 16.

#### IODINE ABSORPTION NUMBER

(All reports should specify method used.)

##### *Hanus Method—Official*

#### 18

##### REAGENTS

(a) *Hanus iodine soln.*—Dissolve 13.2 g of pure I in 1 liter of glacial acetic acid (99.5%) that shows no reduction with dichromate and  $H_2SO_4$ . Add enough Br to double the halogen content as determined by titration (ca 3 ml). The I may be dissolved by heating, but the soln should be cold when Br is added.

A convenient procedure for preparing the Hanus I soln is as follows: Measure 825 ml of acetic acid that has shown no reduction by dichromate test and dissolve in it with the aid of heat 13.615 g of I. Cool, and titrate 25 ml of this soln with the 0.1 N  $Na_2S_2O_3$ . Measure another portion of 200 ml of the acetic acid and add 3 ml of Br. To 5 ml of this soln add 10 ml of the 15% KI soln, and titrate with the 0.1 N  $Na_2S_2O_3$ . Calculate quantity of Br soln required to double halogen content of remaining 800 ml of I soln as follows:

$$A = \frac{B}{C}, \text{ in which}$$

$A$  = ml of Br soln required;

$B$  =  $800 \times$  thiosulfate equivalent of 1 ml of I soln; and

$C$  = thiosulfate equivalent of 1 ml of Br soln.

EXAMPLE: 136.15 g of I is dissolved in 8250 ml of acetic acid, and 30 ml of Br is dissolved in 2000 ml of acetic acid. Titrating 50 ml of the I soln against the standard thiosulfate shows that 1 ml of the I soln = 1.1 ml of the thiosulfate (0.0165 g of I). Titrating 5 ml of the Br soln shows that 1 ml of the Br soln = 4.6 ml of the thiosulfate. Then the quantity of Br soln required to double the halogen content of the

remaining 8200 ml of I soln =  $\frac{8200 \times 1.1}{4.6}$ , or 1961 ml. Upon mixing the two solns

in this proportion, there is obtained a total volume of 10,161 ml, containing 135.3 g of I. In order to reduce this soln to the proper strength (13.2 g of I per liter),

$10.161 \times 13.2 = 134.1$ ;  $135.3 - 134.1 = 1.2$  g of I present in excess, or  $\frac{1.2 \times 1000}{13.2} = 91$  ml

of acetic acid, which must be added.

(b) *Potassium dichromate.*—0.1 N. Dissolve 4.903 g of  $K_2Cr_2O_7$  in  $H_2O$  and dilute to 1 liter. Check strength of this soln against pure Fe.

(c) *Sodium thiosulfate soln.*—0.1 N. Prepare a soln containing 24.82 g of  $Na_2S_2O_3 \cdot 5H_2O$  in freshly boiled and cooled  $H_2O$  and dilute to 1 liter. Standardize this soln as follows: Place in glass-stoppered flask 20 ml of the 0.1 N  $K_2Cr_2O_7$  and 10 ml of 15% KI soln. Add 5 ml of HCl. Dilute with 100 ml of freshly boiled and cooled  $H_2O$  and allow the 0.1 N  $Na_2S_2O_3$  to flow slowly into flask until yellow color of liquid has almost disappeared; add a few drops of starch indicator, VI, 3(e); and with constant shaking continue to add the 0.1 N  $Na_2S_2O_3$  until the blue color just disappears.



## 19

## DETERMINATION

Weigh ca 0.500 g of fat, or 0.250 g of oil (0.100–0.200 g in the case of drying oils that have very high absorbent power), into 500 ml glass-stoppered flask or bottle. Dissolve the fat, or oil, in 10 ml of  $\text{CHCl}_3$ . Add 25 ml of the Hanus I soln and allow to stand for 30 min., shaking occasionally. (This time must be adhered to closely in order to obtain accurate results. The excess of I should be at least 60% of quantity added.) Add 10 ml of 15% KI soln, shake thoroly, and add 100 ml of freshly boiled and cooled  $\text{H}_2\text{O}$ , washing down any free I that may be found on stopper. Titrate the I with the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ , adding it gradually, with constant shaking, until yellow color of the soln has almost disappeared. Add a few drops of starch indicator, VI, 3(e), and continue titration until blue color has entirely disappeared. Toward end of titration, stopper bottle and shake violently, so that any I remaining in soln in the  $\text{CHCl}_3$  may be taken up by the KI soln. Conduct two blank determinations along with that on sample. The number of ml of the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  required by blank less quantity used in determination gives thiosulfate equivalent of the I absorbed by the fat or oil. Calculate percentage by weight of I absorbed and report as the I number (Hanus method).

*Wijs Method—Official*

## 20

## REAGENTS

*Wijs iodine soln.*—Dissolve 13 g of resublimed I in 1 liter of glacial acetic acid (99.5%) and pass in washed and dried Cl gas until original thiosulfate titration of soln is not quite doubled. Use not more than slight excess of I and no excess of Cl. Preserve in glass-stoppered amber bottle sealed with paraffin until ready for use. Do not use Wijs soln that is more than 30 days old. Because of its unstable character  $\text{ICl}_3$  should not be used for preparation of the I soln.<sup>8</sup>

The other reagents and solns used are described under 18.

## 21

## DETERMINATION

Weigh 0.10–0.50 g (depending on I number) of the melted and filtered sample into clean, dry, 16 oz, glass-stoppered bottle containing 15–20 ml of  $\text{CCl}_4$  or  $\text{CHCl}_3$ . With pipet add 25 ml of the I soln, allowing pipet to drain for definite time. The excess of I should be from 50 to 60% of quantity added, that is, from 100 to 150% of quantity absorbed. Moisten stopper with the 15% KI soln to prevent loss of I or Cl but guard against use of quantity sufficient to run down inside bottle. Let bottle stand in dark place for 30 min. at uniform temp. Add 20 ml of 15% KI soln and 100 ml of recently boiled and cooled  $\text{H}_2\text{O}$ . Titrate the I with the 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  soln, adding latter gradually and with constant shaking until yellow color of soln has almost disappeared. Add a few drops of starch indicator, VI, 3(e), and continue titration until blue color has entirely disappeared. Toward end of reaction stopper bottle and shake violently so that any I remaining in soln in the  $\text{CCl}_4$  or  $\text{CHCl}_3$  may be taken up by the KI soln. Conduct two determinations on blanks, run in same manner as sample, but without any fat. Slight variations in temp. affect quite appreciably titer of the I soln as acetic acid has high coefficient of expansion. It is essential, therefore, that blanks and determinations on sample be made at same time. Number of ml of standard thiosulfate soln required by blank less quantity used in determination gives thiosulfate equivalent of the I absorbed by sample taken. Calculate percentage by weight of I absorbed and report as the I number (Wijs method).

## THIOCYANOGEN NUMBER OF FATS AND OILS—TENTATIVE

22

## REAGENTS

(a) *Lead thiocyanate*.—Dissolve 331 g of finest C.P.  $\text{Pb}(\text{NO}_3)_2$  in 700 ml of  $\text{H}_2\text{O}$  and filter. Dissolve 194 g of C.P.  $\text{KCNS}$  in 500 ml of  $\text{H}_2\text{O}$  and filter. Slowly add the  $\text{Pb}(\text{NO}_3)_2$  soln to the  $\text{KCNS}$  soln with stirring, continue stirring for 30 min., and allow precipitate to settle. Decant supernatant liquid thru filter paper on Büchner funnel, using slight suction, and wash precipitate several times with  $\text{H}_2\text{O}$  by decantation. Transfer precipitate to Büchner funnel, using horn spoon and  $\text{H}_2\text{O}$ , and wash with  $\text{H}_2\text{O}$  until washings give no test for nitrates. Place precipitate on watch-glass and dry to constant weight (ca 7 days) in vacuum desiccator over  $\text{H}_2\text{SO}_4$ . The dried  $\text{Pb}(\text{SCN})_2$  should be white in color. Store in air-tight brown bottle and keep in the dark. Yield ca 260 g.

(b) *Thiocyanogen soln*.—Prepare anhydrous acetic acid by boiling gently in liter flask with ground-in glass air condenser, for ca 1 hour, 500 ml of acetic acid, containing at least 99.5% of acetic acid, with 40 ml of acetic anhydride. Attach a  $\text{CaCl}_2$  tube to end of condenser and allow the acid to cool to room temp.

*Solution 1*.—Weigh 4.2 g of dry Br into 250 ml graduated flask, dissolve in 100 ml of pure dry  $\text{CCl}_4$ , and fill flask to mark with anhydrous acetic acid.

*Solution 2*.—Pour 250 ml of anhydrous acetic acid on 12.5 g of the pure dry  $\text{Pb}(\text{SCN})_2$  in a white, dry, glass-stoppered liter bottle.

Add Soln 1 to Soln 2 in small quantities, giving Soln 2 a vigorous shaking after each addition and taking care that decoloration takes place before each addition of soln. After complete mixture of Solns 1 and 2 has been obtained, allow the suspension, consisting of precipitated  $\text{PbBr}_2$  and surplus  $\text{Pb}(\text{SCN})_2$ , to settle. Filter soln thru dry paper into a dry, brown, glass-stoppered bottle. Keep filtrate, which should be clear and colorless, or only slightly yellow, in the dark. This soln, if correctly prepared, will require 24–26 ml of 0.1 *N* thiosulfate soln for its iodometric titration. The thiocyanogen soln will keep ca 1 week. After that time it begins to show a yellow color and a turbidity, and soon a fine yellow precipitate settles to bottom of bottle.

23

## DETERMINATION

Weigh 0.1–0.2 g of the fat or oil (excess of the thiocyanogen reagent should be 100–150%) into 200 ml glass-stoppered bottle or flask. Add 25 ml of the thiocyanogen soln from pipet, rotate bottle gently until fat is dissolved, and allow to stand in the dark 20–24 hours. Add 10 ml of 10%  $\text{KI}$  soln quickly and at one time, while shaking bottle to avoid hydrolysis of the thiocyanogen soln. Add 100 ml of  $\text{H}_2\text{O}$  and titrate the liberated  $\text{I}$  with standardized 0.1 *N* thiosulfate soln in usual manner, using starch indicator. Conduct at least two blank determinations along with the determination on sample. Subtract number of ml of thiosulfate soln required by sample from number required by blank. Multiply this difference by the  $\text{I}$  equivalent of the thiosulfate soln. The value obtained is the quantity of  $\text{I}$  equivalent to the thiocyanogen absorbed by the fat or oil. Calculate the percentage by weight and report as thiocyanogen number.

Using the thiocyanogen number together with the Hanus  $\text{I}$  number, calculate composition of oils or fats composed of glycerides of oleic, linoleic, and saturated acids by following formulas:

$$\begin{aligned}x + y + z &= 100 - \% \text{ unsaponifiable matter;} \\173.3 x + 86.1 y &= 100 \text{ (I No.);} \\86.7 x + 86.1 y &= 100 \text{ (SCN No.), where}\end{aligned}$$

$x = \% \text{ linoleic acid glycerides;}$   
 $y = \% \text{ oleic acid glycerides; and}$   
 $z = \% \text{ saturated acid glycerides.}$

If the percentage of unsaturated acids present in an oil is known, calculate percentages of oleic, linoleic, and linolenic acids present in the oil by following formulas:

$273.7 x + 181.2 y + 89.9 z = 100 \text{ (I No.);}$   
 $182.5 x + 90.6 y + 89.9 z = 100 \text{ (SCN No.);}$   
 $x + y + z = \% \text{ unsaturated acids, where}$   
 $x = \% \text{ linolenic acid in oil;}$   
 $y = \% \text{ linoleic acid in oil; and}$   
 $z = \% \text{ oleic acid in oil.}$

#### SAPONIFICATION NUMBER (KOETTSTORFER NUMBER)—OFFICIAL

24

##### REAGENT

*Alcoholic potassium hydroxide soln.*<sup>9</sup>—(1) Reflux 1.2 liters of alcohol for 30 min. in distilling flask with 10 g of KOH and 6 g of granulated Al (or foil). Distil, and collect 1 liter after discarding first 50 ml. Dissolve 40 g of high-grade KOH in this liter of alcohol. Keep soln in glass-stoppered bottle. Or (2) crush 40 g of high-grade KOH in 7 or 8" mortar. Add 45 g of granulated CaO and grind mixture to powder. From liter of alcohol add 100 ml to mortar and transfer to flask, rinsing mortar with several more portions. Add remainder of alcohol to flask, shake mixture at least 5 min., and invert a beaker over neck of flask. Repeat shaking several times during day. Next morning filter soln into clean, dry, glass-stoppered bottle.

25

##### DETERMINATION

Weigh accurately ca 5 g of filtered sample into 250–300 ml Erlenmeyer flask. Pipet 50 ml of the alcoholic KOH soln into flask, allowing pipet to drain for definite time. Connect flask with air condenser and boil until fat is completely saponified (ca 30 min.). Cool, and titrate with 0.5 *N* HCl, II, 19(a), using phenolphthalein indicator. Conduct blank determination along with that on sample, using same pipet for measuring the KOH soln and draining for same length of time. Subtract number of ml of 0.5 *N* HCl obtained in determination on sample from number obtained on blank to obtain the ml of 0.5 *N* HCl equivalent to the KOH used in saponification of sample taken. Calculate and report as saponification number (mg of KOH required to saponify 1 g of fat).

26

##### SOLUBLE ACIDS—OFFICIAL

Place flask used under 25 and its contents on water bath and evaporate the alcohol. Add that quantity of 0.5 *N* HCl which is equivalent to quantity of KOH used for saponification of the sample under 25 and 1 ml more (quantity of 0.5 *N* HCl to be added = titration for blank – titration for sample + 1 ml), and place flask on steam bath until separated fatty acids form clear layer on upper surface of liquid. Fill flask to neck with hot H<sub>2</sub>O and cool contents in ice H<sub>2</sub>O until cake of fatty acids is thoroly hardened. Pour liquid contents of flask thru filter into liter flask, refill flask with hot H<sub>2</sub>O, and set on steam bath until fatty acids collect at surface. Cool by immersing in ice H<sub>2</sub>O and again filter liquid into the liter flask. Repeat this treatment with hot H<sub>2</sub>O 3 times, cooling and collecting washings in the liter flask after each treatment. Titrate combined washings with 0.1 *N* alkali, using phenolphthalein indicator. Subtract 5 (corresponding to excess of 1 ml of 0.5 *N* acid) from number of ml of 0.1 *N* alkali used and multiply by 0.0088 to obtain weight of soluble acids as butyric acid. Calculate percentage of soluble acids.

27

## INSOLUBLE ACIDS (HEHNER NUMBER)—OFFICIAL

Allow flask containing the cake of insoluble fatty acids from 26 and the paper thru which the soluble fatty acids have been filtered to drain and dry for 12 hours. Transfer cake, together with as much of fatty acids as can be removed from filter paper, to weighed, wide-mouthed beaker flask. Place funnel containing filter in neck of flask and wash paper thoroly with hot absolute alcohol. Remove funnel, evaporate alcohol, dry for 2 hours at  $100^{\circ}$ , cool in desiccator, and weigh. Again dry for 2 hours, cool, and weigh. If there is any considerable decrease in weight, re-heat for 2 hours, cool, and weigh again. Calculate percentage of insoluble fatty acids.

SOLUBLE AND INSOLUBLE VOLATILE ACIDS (REICHERT-MEISSEL AND POLENSKE VALUES)<sup>10</sup>—OFFICIAL

28

## REAGENTS

(a) *Sodium hydroxide soln.*—(1+1). Protect soln from contact with  $\text{CO}_2$ . Allow soln to settle and use only clear liquid.

(b) *Pumice stone.*—Heat small pieces to white heat, plunge into  $\text{H}_2\text{O}$ , and keep there until used.

(c) *Glycerol-soda soln.*—Add 20 ml of the 1+1 NaOH soln to 180 ml of pure concentrated glycerol.

29

## DETERMINATION

Weigh accurately 5 g of sample to be tested into clean, dry, 300 ml flask; add 20 ml of the glycerol-soda soln and heat over flame or asbestos plate until complete saponification occurs, as shown by mixture becoming perfectly clear. If foaming occurs, shake flask gently. Add 135 ml of recently boiled  $\text{H}_2\text{O}$ , dropwise at first to prevent foaming, then add 6 ml of  $\text{H}_2\text{SO}_4$  (1+4) and a few fragments of pumice stone. Distil without previously melting the fatty acids, using apparatus of approximate dimensions illustrated in Fig. 41. Rest flask on piece of asbestos board having a hole 5 cm in diameter in center, and so regulate flame as to collect 110 ml of distillate in as near 30 min. as possible and to allow distillate to drip into receiving flask at temp. not higher than  $18\text{--}20^{\circ}$ .

When distillation is complete, substitute for receiving flask a 25 ml cylinder to collect any drops that may fall after flame has been removed. Mix without violent shaking, immerse flask containing distillate almost completely in  $\text{H}_2\text{O}$  at  $15^{\circ}$  for 15 min., filter the 110 ml of distillate thru dry filter paper 9 cm in diameter, and titrate 100 ml with the standard NaOH soln, using phenolphthalein (1% alcoholic soln) as indicator. The pink color should remain unchanged for 2 or 3 min. The Reichert-Meissel value is the number of ml of 0.1 N NaOH soln used times 1.1, after this result is corrected for figure obtained in blank determination.

Remove remainder of soluble acids from insoluble acids upon filter paper by washing with 3 successive 15 ml portions of  $\text{H}_2\text{O}$ , previously passed thru the condenser, the 25 ml cylinder, and the 110 ml receiving flask. Dissolve the insoluble

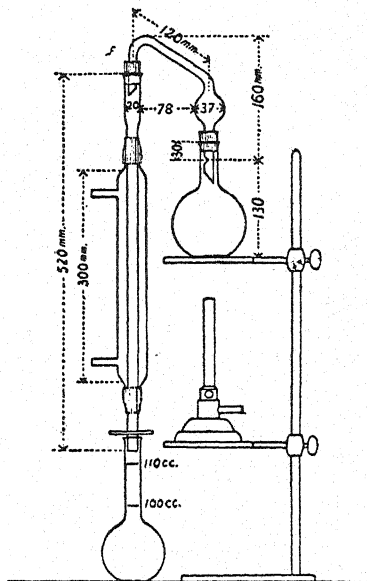


FIG. 41.—APPARATUS FOR DETERMINATION OF POLENSKE NUMBER

acids by passing successive 15 ml portions of neutral alcohol, 95% by volume, thru filter paper, each portion having previously passed thru the condenser, the 25 ml cylinder, and the 110 ml receiving flask. Titrate combined alcoholic washings with the standard NaOH soln, using the phenolphthalein as indicator. The Polenske value equals number of ml of alkali soln required for the titration.

NOTE.—Unless these directions are followed in every detail as described, satisfactory results cannot be obtained.

## 30

KIRSCHNER VALUE<sup>11</sup>—OFFICIAL

To 100 ml of the Reichert-Meissl distillate, 29, in 200 ml Erlenmeyer flask, add 6 drops of phenolphthalein soln and titrate to very faint pink with a 0.1 *N* Ba(OH)<sub>2</sub> soln. Add 0.3 g of finely powdered Ag<sub>2</sub>SO<sub>4</sub>. During next hour shake mixture frequently, filter, and transfer 100 ml of filtrate to 300 ml flask. Add 10 ml of H<sub>2</sub>SO<sub>4</sub> (1+40), 35 ml of H<sub>2</sub>O, and a piece of Al wire or several small pieces of pumice stone, 28(b). Distil 110 ml in ca 20 min., using Polenske apparatus, Fig. 41. Titrate 100 ml of distillate with 0.1 *N* Ba(OH)<sub>2</sub> soln, make blank determination, and after correcting number of ml of alkali used, calculate the Kirschner value according to following formula:  $K = \frac{A \times 121 (100 + B)}{10,000}$ , in which *A* = corrected Kirschner titration and *B* = number of ml of standard alkali soln to neutralize the 100 ml Reichert-Meissl distillate.

Butter fat gives Kirschner values from 19 to 26, coconut oil gives an average of 1.9, and palm kernel oil, 1.0, whereas the majority of other fats and oils give values from 0.1 to 0.2.

## SATURATED AND UNSATURATED FATTY ACIDS

## 31

*Lead Salt-Ether Method*<sup>12</sup>—Official

(Not applicable to fats and oils that contain erucic, elaeostearic, chaulmoogric, hydnocarpic or similar acids; to hydrogenated products that contain notable quantities of iso-oleic acid; nor to coconut or palm kernel oils that contain notable quantities of the lower fatty acids that give ether-soluble Pb salts.)

Weigh accurately 10 or 20 g of sample into 200 ml Erlenmeyer flask. Add 30 ml of alcohol and 8 ml of KOH soln (1+1). Mix thoroly and heat on steam bath for ca 30 min. Add slight excess of acetic acid, using phenolphthalein as indicator, and then add sufficient quantity of 15% KOH soln while rotating flask to produce distinct pink color. Heat to boiling in liter flask 60 ml (120 ml for 20 g of sample) of 20% Pb acetate soln and same quantity of H<sub>2</sub>O. Add the neutralized soap soln cautiously to avoid any loss, rinsing saponification flask with 5 ml of alcohol, then with small volumes of hot H<sub>2</sub>O. Boil mixture gently ca 5 min., shake thoroly, and cool under running H<sub>2</sub>O, rotating flask to cause all precipitated Pb soaps to adhere to sides and bottom of flask. When mixture is cold pour off aqueous soln into large beaker in order to examine soln for particles of Pb soap. (Usually soln is slightly turbid owing to some basic Pb acetate, and no particles or globules of Pb soap are seen.) Wash flask and Pb soap twice with cold H<sub>2</sub>O and allow flask to drain for 10 min. Remove last drops of H<sub>2</sub>O by means of thin roll of filter paper held by forceps, being careful to press paper only lightly against precipitate. Add ca 120 ml of ether and shake by rotating flask ca 5 min.

Connect flask with reflux condenser and boil contents gently until the Pb soap is completely disintegrated or dissolved. Remove flask and rinse down sides with sufficient ether to make final volume ca 150 ml. Invert close fitting beaker over neck of flask and place in icebox for at least 15 hours. Place 7 cm ordinary filter paper

in Büchner funnel of 7.5 cm diameter, turn on full suction, and fit hardened filter paper cut to 8 cm in diameter as snugly as possible to sides of funnel. Decant ether soln from separated Pb soaps, using only sufficient suction to draw ether thru filter. (Too much suction causes ether to evaporate so rapidly that filter may become clogged with separated unsaturated acids, Pb soaps, or ice.)

Transfer precipitate to filter by rinsing flask with small portions of ether. During filtration keep funnel covered as much of the time as possible to prevent evaporation of ether. If at any time filtration proceeds so fast as to cause the mass of Pb soap to crack, close cracks by pressing with small spoon or spatula; otherwise precipitate cannot be properly washed. Rinse spoon free from precipitate and wash precipitate 8 or 10 times with ether, finally allowing suction to continue until precipitate cracks into numerous pieces. Without delay, separate with spoon as much of precipitate as possible and transfer it without loss to 500 ml separatory funnel containing ca 50 ml of ether, washing off any precipitate adhering to spoon and neck of separatory funnel with ether. Transfer filter paper to liter flask. Shake contents of separatory funnel thoroly to disintegrate lumps of Pb salt and allow to stand ca 20 min. Add 20 ml of HCl previously diluted with 10 ml of  $H_2O$  and shake thoroly for 2 min. to decompose all the Pb soap. Add 5-10 ml of HCl (2+1) to the liter flask containing filter paper, shake thoroly to decompose any precipitate adhering to flask and filter; then wash into the separatory funnel with small alternate portions of ether and  $H_2O$  until all fatty acids and  $PbCl_2$  are removed from flask. Again shake contents of separatory funnel with rotary motion and allow to stand for 10 min. Withdraw lower aqueous soln slowly, taking precautions not to remove any emulsion or undecomposed Pb soap. When Pb soap is present (shown in form of lumps that float on top of aqueous soln), add 10 ml of HCl and shake again; add ca 20 ml of  $H_2O$ , shake, and allow mixture to stand until layers have separated. Withdraw aqueous soln and wash ether with successive 25 ml portions of  $H_2O$  until washings are free from HCl. Dehydrate ether with ca 2 g of anhydrous  $Na_2SO_4$  and transfer soln to weighed 300 ml Erlenmeyer flask. Rinse separatory funnel and  $Na_2SO_4$  with several small portions of ether to remove all fatty acids, taking care not to allow any of the  $Na_2SO_4$  to fall into weighed flask. Distil ether, avoiding any loss of the fatty acids, and heat in oven at ca  $110^\circ$  until weight is constant. Obtain weight of saturated acids and save them for later investigation.

Transfer the ether soln of the soluble Pb soaps to 500 ml or 1000 ml separatory funnel, rinsing the Büchner funnel and filter flask with small quantities of ether. Add a mixture of 30 ml of HCl and 75 ml of  $H_2O$  and shake with rotary motion for 2 min. After allowing mixture to stand for 10 min., slowly withdraw the aqueous soln into beaker. If drops of the ether soln are entrapped by the  $PbCl_2$  precipitate and are removed with it, decant soln from the precipitated  $PbCl_2$  that has settled into separatory funnel. Rinse beaker and precipitate with small quantities of ether, adding washings to separator. Rotate contents of separatory funnel and allow to stand for 10 min. Withdraw the aqueous soln and wash ether with successive 50 ml portions of  $H_2O$  until the HCl is removed. Transfer ether soln to 300 ml weighed Erlenmeyer flask. Distil the ether and place flask in oven heated to ca  $110^\circ$  ca 1 hour, while passing stream of  $CO_2$  into flask to prevent oxidation of unsaturated acids. Cool in atmosphere of  $CO_2$ . When cold, remove the  $CO_2$  and weigh. Repeat this treatment until constant weight is obtained.

Determine in duplicate the I numbers of both saturated and unsaturated acid fractions. (The I number of the saturated acid fraction is due to presence of some unsaturated acid.)

To correct for unsaturated acids present in fraction of saturated acids use following formula:

$$\frac{\text{I No. of saturated acid fraction}}{\text{I No. of unsaturated acid fraction}} \times 100 = A \quad \left( \begin{array}{l} \text{percentage of unsaturated acids in} \\ \text{saturated acid fraction} \end{array} \right).$$

Obtain correct value by means of formula  $\frac{A \times B}{100}$ , in which  $B$  is percentage of the impure saturated acids (as found by analysis). Subtract this correction from percentage of impure saturated acids and add it to percentage of unsaturated acids actually determined.

#### FREE FATTY ACIDS IN CRUDE AND REFINED OILS

32

##### *N. C. P. A. Methods—Official*

(a) *In crude oils.*—Weigh 7.05 g of well-mixed oil into 250 ml flask or 4 oz bottle. Add 2 ml of 1% alcoholic phenolphthalein soln to 50 ml of denatured alcohol, SD Formula 30 (1 vol. of methanol and 10 vols. of ethyl alcohol), or isopropyl alcohol, and add sufficient 0.1 *N* NaOH soln to give faint pink color. Add this mixture to the oil in the flask or bottle. Titrate with 0.25 *N* NaOH soln with vigorous shaking until a permanent faint pink color appears and persists for at least 1 min. Report as percentage of free fatty acid expressed as oleic acid. The number of ml of the 0.25 *N* NaOH used in the titration corresponds to the percentage.

(b) *In refined oils.*—Put ca 50 ml of alcohol (Formula 30) into clean, dry 150 ml flask and add a few drops of refined oil and 2 ml of 1% phenolphthalein soln. Place flask in H<sub>2</sub>O at 60–65° until warm, and add a sufficient quantity of the NaOH soln to produce a faint permanent pink color. Weigh 56.4 g of the refined oil into the neutralized alcohol and titrate, occasionally warming and violently shaking mixture until the same faint permanent pink color appears in the supernatant alcohol. Multiply number of ml of 0.1 *N* NaOH by 0.05 and report as percentage of free fatty acid expressed as oleic acid.

#### ACETYL VALUE<sup>13</sup>—OFFICIAL

33

##### *Acetylation*

Boil 50 ml of sample with 50 ml of freshly distilled acetic anhydride under reflux condenser for 2 hours. Pour mixture into 500 ml of H<sub>2</sub>O in beaker and boil for 15 min., while bubbling a stream of air or of CO<sub>2</sub> thru the soln to prevent bumping. Siphon off the H<sub>2</sub>O, add 500 ml more of H<sub>2</sub>O, and boil again for 15 min. Repeat siphoning and boil for 15 min. with a third 500 ml portion of H<sub>2</sub>O. Allow mixture to cool and separate aqueous layer, which should be neutral to litmus. Transfer the acetylated oil to separatory funnel and wash with two 200 ml portions of warm H<sub>2</sub>O. Separate as much of the H<sub>2</sub>O as possible, add 5 g of anhydrous Na<sub>2</sub>SO<sub>4</sub> to the acetylated oil, and let stand for 1 hour, agitating occasionally to assist the drying. Filter thru dry folded filter, preferably in oven heated to 100–110°, and keep filtered oil in oven until it is completely dry. The acetylated product should be a clear, brilliant oil.

34

##### *Saponification*

Weigh accurately 2–2.5 g each of the acetylated oil and of the untreated oil into separate 250 ml Erlenmeyer flasks. Add to each flask exactly 25 ml of alcoholic KOH soln, 24, and reflux for 1 hour. Titrate the warm solns with 0.5 *N* HCl, using phenolphthalein as indicator. Titrate in same way two 25 ml portions of the alcoholic KOH soln. From the mean of these two results, which should be in very close agreement, deduct the volume of the standard HCl required for titration of the acetylated and of the untreated oil, and from results so obtained calculate the saponification num-

ber (mg of KOH required to saponify 1 g of product) of each. Calculate the acetyl value by means of the following formula:

$$A = \frac{S' - S}{1 - 0.00075S}, \text{ in which}$$

$A$  = acetyl value;

$S$  = saponification number of oil; and

$S'$  = saponification number of the acetylated oil.

#### CHOLESTEROL AND PHYTOSTEROL IN MIXTURES OF ANIMAL AND VEGETABLE FATS

35

##### *Alcohol Extraction Method<sup>14</sup>—Tentative*

(Not applicable in presence of hydrogenated soybean oil.)

Introduce 200–300 g of the melted fat into flat-bottomed liter flask. Close neck of flask with 3-holed stopper and insert thru these holes: (1) A reflux condenser; (2) a right-angled glass tube, one arm of which reaches to a point 6 mm above surface of melted fat, the other being closed a short distance from flask by means of short piece of rubber tubing and pinch-cock; (3) a glass tube bent so that one arm reaches to bottom of flask and the other serves as delivery tube for a 700 ml round-bottomed flask containing 500 ml of alcohol.

Place flasks containing melted fat and alcohol on steam bath and heat so that alcohol vapor passes thru the melted fat in the liter flask and is condensed in the reflux condenser, finally collecting in layer over the melted fat. After all alcohol has passed in this manner into flask containing the fat, disconnect flask from which the alcohol has been distilled and attach tube to the short piece of rubber tubing attached to right-angled glass tube, see (2) above, and siphon alcohol layer back into alcohol distillation flask. Reconnect as at first and again distil alcohol as in first operation. When all alcohol has been distilled, siphon it again into distillation flask and extract in same manner a third time.

Discard fat and retain alcohol, which now contains practically all cholesterol and phytosterol originally present in fat. Concentrate alcoholic soln to ca 250 ml, and to boiling liquid add 20 ml of KOH soln (1+1). Boil for 10 min. to insure complete saponification of fat, cool to room temp., and pour into large separatory funnel containing 500 ml of warm ether. Shake to insure thoro mixing and add 500 ml of H<sub>2</sub>O. Rotate funnel gently to avoid formation of extremely stubborn emulsions, but mix the H<sub>2</sub>O thoroly with the alcohol-ether-soap soln. A clear, sharp separation takes place at once. Draw off soap soln and wash ether layer with 300 ml of H<sub>2</sub>O, avoiding shaking. Repeat washing of ether soln with small quantities of H<sub>2</sub>O until all soap is removed. Transfer ether layer to flask and distil ether until volume of liquid remaining in flask measures ca 25 ml. Transfer this residue to tall 50 ml beaker and continue evaporation until all ether is driven off and residue is perfectly dry. If desired, weighed beaker may be used and weight of unsaponifiable matter determined at this point.

Add 3–5 ml of acetic anhydride to residue in beaker, cover beaker with watch-glass, and heat to boiling over free flame. After boiling for a few seconds, remove beaker from flame, cool, and add 35 ml of alcohol, 60% by volume. Mix contents of beaker thoroly, filter off alcoholic soln, and wash precipitate with the 60% alcohol. Dissolve precipitate on filter with stream of hot alcohol, 80% by volume, and wash insoluble portion well with the 80% alcohol. Acetates of cholesterol and phytosterol are dissolved, while the greater portion of impurities present (including paraffin and paraffin oil) remains on filter. Cool combined filtrate and washings to temp. of 10–12° and allow to stand at that temp. for 2–3 hours. During this time



the acetates of cholesterol and phytosterol crystallize from the soln. Collect the crystals upon filter, wash with cold alcohol, 80% by volume, and then dissolve in minimum quantity of hot absolute alcohol. Collect the alcoholic soln of the acetates in small glass evaporating dish, add 2 or 3 drops of  $H_2O$  to soln, and heat if not perfectly clear. Allow alcohol to evaporate spontaneously, stirring contents of dish occasionally to mix deposit of crystals that form upon edges with main body of liquid. As soon as a good deposit of crystals has formed, collect them upon hardened filter; wash twice with cold alcohol, 90% by volume; and dry by suction, drying finally at  $100^\circ$  for 30 min. Determine melting point in apparatus shown in Fig. 40, using  $H_2SO_4$  in outer beaker and glycerol in inner tube.

The melting point of first crop of crystals usually gives definite information as to presence or absence of phytosterol, but the conclusion indicated should be confirmed by recrystallizing the crystals from absolute alcohol and again determining melting point. If crystals are pure cholesteryl acetate, the melting point of second crop should agree closely with that of first. If phytosteryl acetate is present, however, a higher melting point will be noted, as phytosteryl acetate is less soluble in alcohol than cholesteryl acetate. The melting point of cholesteryl acetate is  $114^\circ$ ; that of phytosteryl acetate,  $125$ – $137^\circ$ .

36

*Digitonin Method<sup>15</sup>—Tentative*

Shake vigorously 50 g of the oil, or fat, for 15 min. in separatory funnel with 20 ml of a 1% soln of digitonin in 95% alcohol. Allow mixture to stand for a time until emulsion separates. The lower or fat layer should be quite clear while the alcohol layer contains a bulky, flocculent precipitate. Draw off as much of fat as possible, avoiding any loss of precipitate. Add 100 ml of ether to alcohol layer and filter mixture. After drying in air wash precipitate with ether until free from fat, transfer to tall 50 ml beaker, and add 2–3 ml of acetic anhydride. Cover beaker with watch-glass. Boil slowly over low flame for 30 min. After cooling, add 30–35 ml of alcohol, 60% by volume, and mix contents of beaker thoroly. Filter the alcohol soln. Wash precipitate with the 60% alcohol, then dissolve on filter with stream of hot alcohol, 80% by volume, from wash bottle, and set filtrate aside in cool place ( $10^\circ$  or below). After acetates have crystallized out of this soln filter them off, recrystallize from absolute alcohol, dry, and determine melting point of each crop of crystals as directed under 35.

## UNSATURATED RESIDUE

*F. A. C. Method<sup>16</sup>—Official*

37

## REAGENT

*Petroleum benzin.*—Redistil below  $75^\circ$ . Make blank determination by evaporating 350 ml of the reagent with ca 0.25 g of stearine or other hard fat (previously brought to constant weight by heating) and drying as in actual determination. The blank must not exceed a few mg.

38

## APPARATUS

*Extraction cylinder.*—Glass-stoppered, graduated at 40 ml, 80 ml, and 130 ml, and of following dimensions: diameter ca  $1\frac{3}{8}$ ", height ca 12".

39

## DETERMINATION

Weigh 5 g ( $\pm 0.020$  g) of sample into a 200 ml Erlenmeyer flask, add 30 ml of redistilled approximately 95% alcohol (by volume) and 5 ml of 50% aqueous KOH,

and boil mixture for 1 hour under reflux condenser. Transfer to extraction cylinder and wash to 40 ml mark with redistilled 95% alcohol. Complete transfer, first with warm, then with cold  $H_2O$ , until total volume is 80 ml. Rinse flask with 50 ml of petroleum benzin and add rinsings to contents of cylinder previously cooled to room temp. Shake as vigorously as possible for 1 min. and allow to settle until both layers are clear, when volume of upper layer should be ca 40 ml. Draw off petroleum benzin layer as closely as possible by means of slender glass siphon into separatory funnel of 500 ml capacity. Repeat extraction at least 6 more times, using 50 ml of petroleum benzin for each extraction. Wash combined extracts in separatory funnel three times with 25 ml portions of 10% alcohol by volume, shaking vigorously each time. Transfer the petroleum benzin extract to weighed Erlenmeyer flask and distil; or, if desired, evaporate petroleum benzin on steam bath in current of air. Heat flask with residue until constant weight is obtained in oven at uniform temp. not less than  $100^\circ$  nor more than  $110^\circ$ . (A vacuum oven may be used at a corresponding temp., which depends upon pressure used in it. It is important to displace with air any residue vapors of petroleum benzin remaining in flask after heating and before it is weighed.) Deduct any blank from weight before calculating unsaponifiable matter. Test final residue for solubility in 50 ml of petroleum benzin at room temp. Filter, and wash free from insoluble residue, if any. Evaporate and dry in same manner as before.

#### RESIN OIL

40

##### *Qualitative Test—Tentative*

Polarize the pure oil, or a definite dilution with petroleum benzin, in 200 mm tube. Resin oil has a polarization in 200 mm tube of from  $+30^\circ$  to  $+40^\circ$  on sugar scale (Schmidt and Haensch), while most oils<sup>17</sup> read between  $+1^\circ$  and  $-1^\circ$ .

#### COTTONSEED OIL

41

##### *Halphen Test<sup>18</sup>—Official*

Mix  $CS_2$  containing 1% of S in soln with an equal volume of amyl alcohol. Mix equal volumes of this reagent and sample under examination and heat in bath of boiling, saturated brine for 1–2 hours. In presence of as little as 1% cottonseed oil, a pronounced characteristic red or orange-red color is produced. The depth of color is proportional, to a certain extent, to quantity of cottonseed oil present, and comparative tests with known mixtures of cottonseed oil will give an approximation of quantity.

Different oils react with different intensities. Oils that have been heated to  $200$ – $210^\circ$ <sup>19</sup> react with greatly diminished intensity. Heating for 10 min. at  $250^\circ$  renders cottonseed oil incapable of giving the reaction.<sup>20</sup> The fat of animals fed on cottonseed meal or other cottonseed products may give a positive reaction by this test.

42

#### PEANUT OIL<sup>21</sup>—OFFICIAL

Weigh 20 g of the oil into Erlenmeyer flask. Saponify with alcoholic KOH soln, 24; neutralize exactly with acetic acid (1+3), using phenolphthalein indicator; and wash into an 800–1000 ml flask containing boiling mixture of 100 ml of  $H_2O$  and 120 ml of 20% Pb acetate soln. Boil for a minute and then cool precipitated soap by immersing flask in  $H_2O$ , occasionally giving it a whirling motion to cause soap to stick to sides of flask. After flask has cooled, decant the  $H_2O$  and excess of Pb acetate soln and wash the Pb soap with cold  $H_2O$  and alcohol, 90% by volume. Add 200 ml of ether, cork, and allow to stand until soap is disintegrated; heat on water bath, using reflux condenser, and boil ca 5 min.<sup>22</sup> In the case of oils, most of the soap

will be dissolved, while in lards, which contain much stearin, part of soap will be left undissolved. Cool ether soln of soap to 15–17° and allow to stand until all insoluble soaps have separated out (ca 12 hours).

Filter upon Büchner funnel and thoroly wash insoluble Pb soaps with ether. Wash ether-insoluble Pb soaps into separatory funnel by means of jet of ether, alternating at end of operation if a little of the soap sticks to paper with HCl (1+3). Add sufficient HCl (1+3) so that total volume of acid amounts to ca 200 ml and enough ether to make its total volume 150–200 ml and shake vigorously for several minutes. Allow layers to separate, run off acid layer, and wash ether once with 100 ml of the dilute HCl and then with several portions of H<sub>2</sub>O until H<sub>2</sub>O washings are no longer acid to methyl orange. If a few undecomposed lumps of Pb soap remain (indicated by solid particles remaining after third washing with H<sub>2</sub>O), break these up by running off almost all water layer, adding a little HCl and shaking; then continue washing with H<sub>2</sub>O as before. Distil the ether from soln of insoluble fatty acids and dry latter in flask by adding a little absolute alcohol and evaporating on steam bath. Dissolve dry fatty acids by warming with 100 ml of 90% alcohol by volume. Cool slowly to 15°, shaking to aid crystallization. Allow to stand at 15° for 30 min. In presence of peanut oil, crystals of arachidic acid will separate from the soln. Filter, and wash precipitate twice with 10 ml of alcohol, 90% by volume, and then with alcohol, 70% by volume, taking care to maintain arachidic acid and wash solns at definite temp. in order to apply solubility corrections given below. Dissolve arachidic acid upon filter with boiling absolute alcohol, evaporate to dryness in weighed dish, dry, and weigh. Add to weight 0.0025 g for each 10 ml of 90% alcohol used in crystallization and washing, if conducted at 15°; if conducted at 20°, add 0.0045 g for each 10 ml. The melting point of arachidic acid thus obtained is 71–72°. Twenty times weight of arachidic acid will give approximate quantity of peanut oil present. Arachidic acid has characteristic appearance and may be identified under microscope. As little as 5–10% of peanut oil can be detected by this method.

## 43

COLD TEST<sup>23</sup>—TENTATIVE

(Applicable to refined wintered salad oils.)

Fill 4 oz sample bottle with the oil at temp. of 25°, insert cork stopper tightly, and seal with paraffin. Submerge bottle completely in bucket containing finely cracked ice and add H<sub>2</sub>O until it rises to top of bottle. Keep bucket filled solidly with the ice by removing any excess H<sub>2</sub>O and adding ice when necessary. At end of 5 hours remove bottle and examine oil. If it is properly wintered, sample will be brilliant, clear, and limpid.

## 44

DETECTION AND ESTIMATION OF TEA SEED OIL IN OLIVE OIL<sup>24</sup>—OFFICIAL

For preliminary qualitative test use following room temp. method: Measure into test tube (18×150 mm is convenient size) exactly 0.8 ml of acetic anhydride, 1.5 ml of CHCl<sub>3</sub>, and 0.2 ml of H<sub>2</sub>SO<sub>4</sub>. Mix, and cool to room temp. Add directly to reagents 7 drops of the oil to be tested, mix, and cool again. (To measure the 7 drops of oil use glass tubing, 4 mm outside diameter, and ca 2 mm inside diameter. These 7 drops should weigh ca 0.22 g.) If solution of oil in reagents is cloudy after mixing and cooling, add acetic anhydride dropwise, shaking after each addition until a clear solution is suddenly formed. Appreciable deviations from these quantities, particularly in the H<sub>2</sub>SO<sub>4</sub>, cause distinct variations in color intensities. Since the mixed reagent deteriorates slowly do not mix in advance of testing.

After test tube and contents have remained at room temp. for 5 min., note color produced. Tea seed oil will exhibit a deep green by reflected light and brown by

transmitted light. Olive oil will show green color by reflected and transmitted light, occasionally exhibiting a faint fluorescence. Add 10 ml of anhydrous ethyl ether from graduated cylinder and mix immediately by inverting once. Tea seed oil will show brown color changing to intense red within a minute or so. This red color reaches a maximum and then fades slowly within a few minutes. Olive oil forms an initial green color on addition of the ether. This color fades slowly to brown-gray, occasionally passing thru faint pink stage. Both olive oil and tea seed oil will eventually fade to a permanent light brown color. Mixtures of tea seed oil and olive oil show the characteristic tea seed oil colors proportional in intensity to quantity of tea seed oil present.

For approximately quantitative estimations drop oil into reagents as described above and allow to remain at room temp. for 5 min. In the meantime, cool 10 ml portion of anhydrous ethyl ether in ice  $H_2O$ . At end of 5 min. period, place test tube containing oil and reagents in the ice  $H_2O$  for 1 min., add the cold ether (taking care that no  $H_2O$  falls into test tube), and mix. Return tube to ice water bath and allow colors to develop while it is immersed in the ice  $H_2O$ . The colors will develop slowly and reach a maximum within 5 min. This maximum intensity will remain stable for 5-10 min.

Use deepest red colors produced as basis for comparison, and because of short period of stable maximum intensity do not test more than three oils at one time. Standards containing known quantities of tea seed oil in an olive oil that gives little or no pink color with this test should be run simultaneously with sample. The preliminary room temp. test will give indication of standards to be used in the ice-water method.

#### SESAME OIL

45

##### *Baudouin Test—Official*

Dissolve 0.1 g of finely powdered sugar in 10 ml of  $HCl$ , add 10 ml of the sample, shake thoroly for 1 min., and allow to stand for 10 min. In presence of even a very small admixture of sesame oil, the aqueous soln is colored crimson. It should be observed that some olive oils, especially those of African or Spanish origin, give pink or crimson colors. These can be readily differentiated from the color due to sesame oil by the modified Villavecchia test, 46.

46

##### *Modified Villavecchia Test<sup>25</sup>—Official*

Add 2 ml of furfural to 100 ml of alcohol. Mix thoroly 0.1 ml of this soln with 10 ml of  $HCl$  and 10 ml of the sample by shaking them together in test tube for 15 seconds. Allow mixture to stand for 10 min., observe color, add 10 ml of  $H_2O$ , shake, and again observe color. If the crimson color disappears, sesame oil is not present. (As furfural gives a violet tint with  $HCl$ , it is necessary to use the very dilute soln specified.)

#### 47 DETECTION OF FOREIGN FATS CONTAINING TRISTEARIN IN LARD<sup>26</sup>—TENTATIVE

Weigh 5 g of the melted and filtered lard into glass-stoppered cylinder and add 20 ml of warm acetone. Mix well, taking care that soln is clear and has temp. above  $30^\circ$ . Let stand at constant temp. of  $30^\circ$  for 16-18 hours. A fine mass of crystals occupying a volume of not more than 3 ml should then be found at bottom of cylinder. Should volume of crystals materially exceed 3 ml, take smaller quantity of lard (3-4 g) for new test. Should no crystals be deposited, as may be the case with soft or oily lard, absence of tristearin is indicated. Decant supernatant acetone soln from the crystallized glycerides. Add warm ( $30$ - $35^\circ$ ) acetone in three portions of

5 ml each from small wash bottle, taking care not to break up deposit in washing, and decant first two portions. Actively agitate third portion in the cylinder, and by a quick movement transfer crystals to small filter paper. Using wash bottle, wash crystals with 5 successive small portions of the warm acetone and remove excess acetone by suction. Spread out the paper and its contents, breaking up any large lumps and allow to dry in air at room temp. Thoroughly comminute the mass and take melting point of crystals in a closed 1 mm tube, using apparatus similar to that indicated under 15. Heat the  $H_2O$  in the beaker rapidly to ca  $55^\circ$  and maintain this temp. until thermometer carrying melting point tube registers  $50^\circ$ , then heat again and raise temp. of outer bath rather quickly to  $67^\circ$ . Remove burner. The melting point is reached when the fused substance becomes perfectly clear and transparent. When melting point of glycerides obtained by this method is below  $63.6^\circ$  the presence of beef fat or other fat containing tristearin should be suspected, and a melting point of  $63.2^\circ$  or lower is evidence that sample is not pure lard. It is advisable to carry out method with control sample of pure lard. If the melting point of the glycerides, plus twice the difference between the melting point of the glycerides and the melting point of the fatty acids, is less than  $73^\circ$ , the lard is regarded as adulterated.

The conclusion indicated by melting point may be confirmed by taking melting point of the fatty acids prepared from the glycerides. After determining melting point, transfer crystallized glycerides to 50 ml beaker, add 25 ml of ca 0.5 *N* alcoholic KOH, and heat on steam bath until saponification is complete. Pour soln into separatory funnel containing 200 ml of  $H_2O$ , acidify, add 75 ml of ether, shake, and let stand. Draw off aqueous acid layer and wash ether soln at least 3 times with  $H_2O$ . Transfer ether soln to clean dry 50 ml beaker, volatilize ether on steam bath, and finally dry acids at  $100^\circ$ . After ca 2 hours, determine melting point.

Conclusions may be confirmed further by precise determinations of the mean molecular weight of the separated fatty acids. Dissolve the acids in colorless, redistilled alcohol that has been carefully neutralized immediately before use with 0.5–0.2 *N* KOH soln. If sample is pure lard, the mean molecular weight of the fatty acids should correspond closely to that of the fatty acids of  $\alpha$ -palmito-distearin, 274.67. If sample is impure, the mean molecular weight should tend to approach that of the fatty acids from tristearin, 284.

#### FISH OIL AND MARINE ANIMAL OILS IN PRESENCE OF VEGETABLE OILS AND IN ABSENCE OF METALLIC SALTS

48

##### *Qualitative Test—Tentative*

Using test tube dissolve ca 6 g of sample in 12 ml of mixture of equal parts of  $CHCl_3$  and glacial acetic acid. Add Br, dropwise, until slight excess is indicated by color, keeping soln at ca  $20^\circ$ . Allow mixture to stand 15 min. or more and place test tube in boiling  $H_2O$ . If vegetable oils only are present, the soln will be perfectly clear, but fish oils will remain cloudy owing to presence of insoluble bromides.

49

#### COLORING MATTERS—TENTATIVE

Into each of four 500 ml separators measure out four 100 ml portions of sample and dilute each funnel with 100 ml of low-boiling gasoline. Extract two or three times with 50 ml portions of 2 *N*  $Na_2CO_3$ , passing same successively thru each funnel. If yellow or pink color is obtained, test for Sudan G, annatto, or turmeric. Extract the oil soln successively with three 50 ml portions of mixture consisting of HCl and glacial acetic acid (1+5). A pink or red lower layer indicates aniline yellow (7) [15], butter yellow (16) [19], yellow AB (–) [21], or yellow OB (–) [61]. For detailed separation see **XXI**.

COTTONSEED<sup>27</sup>—OFFICIAL

## SAMPLING

50

## EQUIPMENT

(a) *Trier*.—Corkscrew-type that will take ca 4 lbs. of cottonseed at a probe. Made of band steel 7/16×5/32" bent to form an open cylinder ca 3" in diameter, the pitch of the twist being ca 2" and the screw portion being ca 42" long. A single, slight twist will cut the walls so that the trier may be withdrawn readily.

(b) *Receptacles*.—Made by attaching an 8×5×5½" elevator bucket to a pole long enough to enable sampler to place bucket in level position near top of seed chute while standing outside of car and directly in front of it.

(c) *Shaker cleaner*.—Motor-driven. Has screens 3×7' and is provided with adjustable by-passes for securing a representative cross-section sample of seed for analysis.

(d) *Metal containers*.—With close-fitting covers 2½ bu. capacity.

(e) *Friction top cans*.—155 cu. in. capacity. For sending samples to chemist.

51

## PROCEDURE

(a) *Car lots, before unloading*.—By means of trier, draw portions of the seed at different points in each end and in middle of car, taking, in all, not less than 10 portions. If car is so filled with seed that the trier cannot be used, divide contents into 4 sections. In the center of each section dig a hole 30" deep, using a short handled, 5-tine fork. From sides and bottom of each hole take ca 15 lbs of seed with the fork. Place portion of seed taken at each hole in a strong moisture-proof bag. Directly after sampling, collect and remove the four bags from car, empty into one of the metal sample containers, and replace cover.

(b) *Car lots, during unloading*.—Place the elevator bucket attached to a pole in center of unloading chute at regular intervals (depending upon rate of unloading) so that ca 2 lb portions are taken for each ton of seed ejected from the car. (Whether drawn before or during unloading, the several portions collected from car lots should total not less than 50 lbs in weight.)

(c) *Truck or wagon loads, before unloading*.—Insert trier in the load at not less than 5 points. If load is so deep that trier does not reach bottom, fork the seed away from three different parts of load and with the trier withdraw three additional portions of seed from the bottom, making a total of eight portions of seed. Weight of the sample taken should not be less than 2 lbs for each ton of seed in load.

Immediately place each portion of original sample in a properly identified metal container and cover promptly. Weigh entire sample and pass it over a shaker cleaner. Collect and weigh all separated foreign matter or the cleaned seed. Calculate percentage of foreign matter.

If the shaker cleaner is not equipped with a sample reducing device, place all cleaned seed in an efficient mixer (MacLellan Mixer, Model OCS, Serial No. 806). After mixing thoroly, discharge seed, and without any further mixing take a 2 lb sample for analysis, putting it without delay in a friction-top can. Also place inside can a report giving weights of original sample, cleaned seed, and foreign matter. Do all cleaning, mixing, and handling of samples expeditiously in order that they neither lose nor gain moisture.

52

## FOREIGN MATTER

Examine laboratory sample immediately and if found not to have been thoroly cleaned carefully weigh and reclean by use of 6-mesh screen and by the hand-picking

of all remaining particles of foreign matter. Calculate percentage of foreign matter by dividing the weight of the foreign matter reported by sampler by the weight of the original sample and correcting result by adding the percentage of foreign matter found in the laboratory sample.

## 53

## MIXING AND QUARTERING

Mix the cleaned laboratory sample and quarter by one of following methods:

(a) Place sample in approved mechanical mixer (MacLellan Mixer No. 00-S) and mix by revolving 10 times at rate of 5 revolutions a minute. After mixing, empty sample onto large piece of paper, press, and quarter with large spatula. Separate quadrants Nos. 1 and 3 from quadrants Nos. 2 and 4. Immediately return quadrants Nos. 1 and 3 to original container, seal, and retain as referee sample. Preserve quadrants Nos. 2 and 4 in air-tight container for analysis. When finally analyzing them again place on piece of paper and requarter until the combining of opposite quadrants yields two samples of ca 120 g each. Use one of these samples for the determination of free fatty acids. Further quarter the other sample to yield two samples of ca 60 g each, using one of these for moisture determination and the other for oil and ammonia determinations.

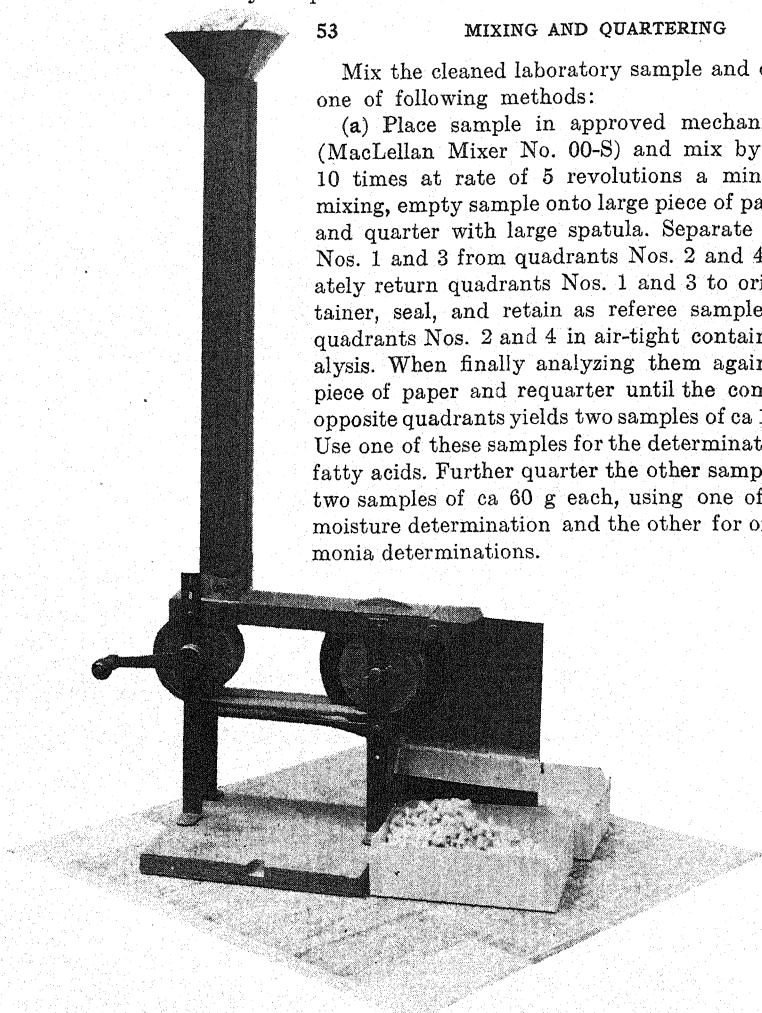


FIG. 42.—MELOY COTTONSEED MIXER AND DIVIDER

(b) Mix and quarter laboratory sample by passing entire sample thru the Meloy cottonseed sample divider, Figs. 42 and 43. (A perfect mix can be accomplished by passing entire sample thru divider 2 or 3 times.) After mixing, reserve one of the two portions resulting from the division, of ca 500 g each, as referee sample. Again pass second portion thru divider. Reserve one of the resulting two portions, of ca 250 g each, for emergency check analysis. Return second portion thru divider and use one of resulting portions, of ca 125 g each, for determination of free fatty acids. Again divide remaining portion to yield two portions of ca 60 g each and use one

portion for the moisture determination and the other for the oil and ammonia determinations.

54

## MOISTURE

Weigh into shallow moisture dishes as rapidly as possible two portions of 8–10 g each of the whole seed and distribute evenly. Place uncovered dish in an approved forced-draft circulating oven at  $101^{\circ}$  for 12–16 hours, or overnight. Remove dish from oven, cover, cool in efficient desiccator 30 min., and weigh. Calculate loss in weight as moisture.

55

## PREPARATION OF SEED FOR OIL AND AMMONIA DETERMINATIONS

Dry the approximately 60 g portion, quartered out for the purpose, for 2 hours at  $130^{\circ}$ ,  $\pm 3^{\circ}$ , in approved type of uniform forced-draft circulatory oven. Absorb into inner walls and bottom of porous earthenware vessel (such as 3" flower pot) 1.5 ml of HCl. (Acid should be well distributed over sides and bottom of pot. When acid has been absorbed the pot should appear dry; if it does not it was probably not in proper condition for this use.)

Cover pot containing seed with watch-glass and place in fuming oven (well-ventilated non-corrodible oven capable of reaching and maintaining temp. of  $115^{\circ}$ ), previously opened and ventilated at least 5–10 min., and fume for 1 hour. (The oven temp. should gradually rise to, but not exceed,  $115^{\circ}$ . After fuming, the lint should be loose and brittle but not scorched.) Grind treated seed in a Bauer mill (No. 148 laboratory mill with No. 6912 plate), which has been adjusted to produce a fine meal. After grinding, open mill and carefully brush out all remaining meal onto a sizable smooth sheet of paper. (It is important that top of hopper of the Bauer mill be fitted with a cover to prevent loss of seed during grinding.) If loss exceeds 1 g, repeat entire process as the lost material is not necessarily representative of the whole portion.

Mix ground sample thoroly, preferably by placing it in 2 quart Mason fruit jar together with large rubber stopper. Replace cover and shake violently until ground material is thoroly mixed; transfer to well-stoppered bottle or container of just sufficient size to hold material tightly so as to prevent percolation or vertical segregation of the components.

56

## MOISTURE IN GROUND SAMPLE

Weigh 5 g of the fumed and ground sample into moisture dish and dry at  $101^{\circ}$  for 2 hours in oven specified in 54. Calculate loss in weight as moisture content.

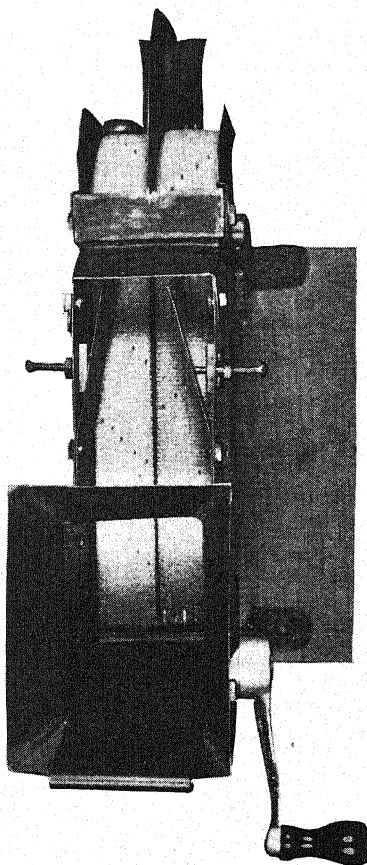


FIG. 43.—TOP VIEW OF MELOY DIVIDER SHOWING ADJUSTMENT OF BAFFLES



## OIL

57

## APPARATUS

- (a) *Extractor*.—Butt type.  
 (b) *Condensers*.—Allihn's with 12" jackets, fitted with cork connections.

58

## REAGENT

*Petroleum benzin*.—Initial boiling temp., 35–38°; dry-flask end point, 52–60°; at least 95% distilling under 54°, and not over 60% distilling under 40°; sp. gr. at 60°F., 0.630–0.660; color, water white; evaporation residue, not over 0.0011 g; doctor test, sweet; copper-strip corrosion test, noncorrosive; trace only of unsaturated compounds permitted; residue in distilling flask, neutral to methyl orange; blotter strip odor test, odorless within 12 min.; aromatic compounds, no nitro-benzene odor; saponification value, less than 1.0 mg KOH per 100 ml.

Make distillation test according to method of American Society for Testing Materials (standard method D216–232) and make a blank by evaporating 250 ml with ca 0.25 g of stearin or other hard fat (previously brought to constant weight by heating) and drying as in actual determination. The blank must not exceed a few mg.

59

## DETERMINATION

Weigh accurately duplicate samples of 4–5 g of the fumed and ground seed, 55, and spread each portion in thin layer on 150 mm filter paper (S & S No. 597 or equivalent grade); fold paper over sample at a point ca one-quarter distance from each of two opposite sides to center; wrap by coiling from one of unfolded sides into cylinder; and rewrap in second paper or papers in such manner as to prevent escape of the meal, leaving top of second paper open like a thimble. Place piece of absorbent cotton in top of thimble to distribute the dropping benzin. Place 25 ml of the petroleum benzin in tared flask, 125 ml capacity, and extract sample for 4 hours. (The benzin should drop on center of thimble at a rate of at least 150 drops per min., and volume of solvent should be kept about constant.) Evaporate solvent until no trace remains, cool sample to room temp., and weigh. As the last traces of benzin are sometimes difficult to detect by odor, in case of doubt heat for an hour, or longer, until constant weight is obtained. Calculate oil content as shown in the following example:

Petroleum benzin extract.....	1.025 g
Original moisture plus total foreign matter up to and including 1.0%.....	12.2 + 0.8 = 13 0%
Second moisture.....	2.6
Total foreign matter up to and including 1.0%...	0.8%
Weight of sample.....	5.00 g
$\frac{1.025}{5} \times \frac{87}{97.4} = 18.3\% \text{ of oil in original seed.}$	

60

## AMMONIA

Digest 1.7034 g of sample in 650–800 ml Kjeldahl flask with ca 0.5 g of metallic Hg or 0.7 g of HgO, 10 g of Na<sub>2</sub>SO<sub>4</sub> or K<sub>2</sub>SO<sub>4</sub>, and 25 ml of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84). Place flask in inclined position and heat below boiling point of the acid from 5 to 15 min., or until frothing has ceased. Increase temp. and continue digestion until liquid becomes colorless, or until complete digestion is obtained. From this point use the regular Kjeldahl method, omitting addition of KMnO<sub>4</sub>.

After cooling mixture add ca 300 ml of H<sub>2</sub>O, a few granules of Zn to keep contents

of the flask from bumping, and 25 ml of the  $K_2S$  or  $Na_2S_2O_3$  soln, or sufficient quantity to precipitate all the Hg. After mixing thoroly, add 60 ml of caustic soda soln (sp. gr. 1.50), or sufficient to make strongly alkaline, pouring soln down side of flask so that it does not mix at once with the acid soln. Connect flask with condenser of block tin, mix contents of flask by shaking, and distil into an accurately measured quantity of standard  $H_2SO_4$  soln (0.5 *N*) to which has been added 50 ml of  $H_2O$ , until at least 200 ml of distillate is obtained, taking care that delivery tube reaches below level of the standard acid. Add ca 1 ml of 0.2% aqueous soln of Na alizarin sulfonate as the indicator. (Either cochineal or methyl red may be used as indicator, but with methyl red the soln is titrated hot.) Titrate distillate with a standard 0.25 *N* NaOH soln.

By using 1.7034 g of sample for the analysis, the number of ml of 0.5 *N* acid required for neutralization of the distilled  $NH_3$ , divided by 2, gives percentage of  $NH_3$ .

Make blank test on all reagents and correct titration of above distillate accordingly.

61

## CALCULATION

Example:

	ml
0.5 <i>N</i> $H_2SO_4$ measured into flask.....	10.00
0.5 <i>N</i> $H_2SO_4$ for blank test on reagents.....	0.06
0.25 <i>N</i> NaOH used in titration.....	2.68

$$\frac{10 - 0.06}{2} - \frac{2.68}{4} = 4.30\% \text{ ammonia in fumed seed.}$$

Original moisture.....	8.1
Moisture in fumed seed.....	2.0
Foreign matter, up to 1.0.....	0.9

$$\frac{4.30 \times 0.91}{0.98} = 3.99\% \text{ ammonia in original seed.}$$

62

## FREE FATTY ACIDS

Heat 200 g of original clean sample of seed for 30–40 min. at temp. of 100–105°, and cool. Pass the cooled seed thru Bauer mill set to merely crack all the seed. Separate meats from hulls by use of 4–6-mesh screen. Grind meats in Ruswine No. 1 food chopper equipped with 16-tooth blade. Thoroly mix sample and pass thru 15-mesh screen so as to remove any remaining lint or hulls. (Proper grinding and complete separation of meats from hulls are essential points in obtaining concordant results.) Without undue loss of time quarter the thoroly mixed ground meats so as to obtain at least a 40 g sample. Extract this sample by cold percolation in following manner: Place lower disk from a Knorr extraction apparatus in a Butt tube and place on it a layer of asbestos fiber suspended in petroleum benzin. (A satisfactory mat should allow none of meats to pass thru but should allow extracting solvent to flow thru at ca 150 drops per min.) Place sample in prepared tube, and add 50 ml of petroleum benzin followed by two portions of 25 ml each of petroleum benzin, allowing each portion to flow thru before adding next portion. Allow extracted oil to remain on steam bath for 1½ hours to completely remove all trace of the solvent. Weigh 7.05 g of the oil into titration flask; add 30 ml of neutralized alcohol (SD Formula 30, VIII, 2,) or isopropyl alcohol and 1 ml of 1% phenolphthalein (10 ml of petroleum benzin may be added if necessary); and titrate free fatty acid with 0.25 *N* alkali. Shake flask vigorously during titration, and take as end point a permanent pink that persists at least 1 min.

$$\% \text{ F.F.A.} = \frac{28.2 \times \text{normality of alkali} \times \text{ml used}}{\text{weight of oil}}$$

If results indicate a free fatty acid content of 4% or higher, duplicate the complete test.

#### OIL IN FLAXSEED<sup>28</sup>

##### *Refractometric Method—Official*

63

#### PREPARATION OF STANDARD SOLVENT

Prepare a mixture of ca 74% halowax and 26%  $\alpha$ -bromonaphthalene by weight, and carefully adjust composition of mixture to refractive index of 1.63940 at 25.0°.

(If a temp. regulating device is available the determination of refractive index is simplified by passing H<sub>2</sub>O at exactly 25.0° thru the water jacket of the refractometer. Equally satisfactory results may be obtained, however, by using H<sub>2</sub>O at room temp. and making necessary correction. For the above mixture this correction in refractive index is 0.00045 per 1°, to be added to reading if temp. is above 25.0° and subtracted if temp. is below that point. It is important that all water-jacket temp. readings be made to nearest 0.1°.)

Keep soln in glass or Pb-stoppered dark bottle and away from direct sunlight. (The refractive index should keep constant for a long period of time, but it is advisable to check it from time to time.)

#### 64 PREPARATION OF SAMPLE

Obtain representative sample of ca 25 g of the clean seed either by hand quartering original sample or by use of a mechanical sampling device. Grind material to such degree of fineness that after extraction with ether 95% of sample will pass thru

40-mesh sieve. (A motor-driven experimental roller flouring mill with 6×6" rolls, 40 corrugations to the inch, has been found satisfactory. The rolls should have a speed differential of 9:7 and the faster roll should have a speed of ca 900 r.p.m.) (See Fig. 44.)

65

#### DETERMINATION

Weigh out accurately 2.5 g of finely ground, well-mixed sample and transfer to clean 3" porcelain mortar that has been previously heated to ca 70° in oven or on

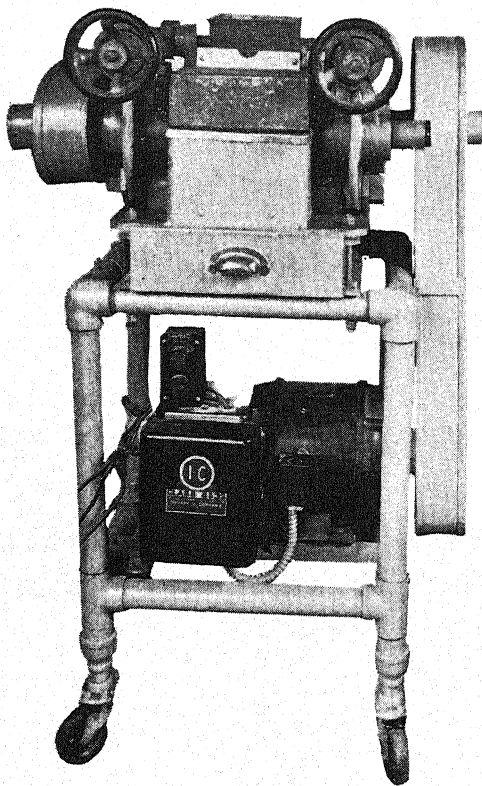


FIG. 44.—ROLLER-TYPE EXPERIMENTAL FLOURING MILL SUITABLE FOR GRINDING FLAXSEED SAMPLES

electric hot plate at low heat. Add ca 1 g of reagent quality sea sand or similar abrasive and exactly 5 ml of the standard mixture of halowax and  $\alpha$ -bromonaphthalene. (Since this mixture has a high sp. gr. it is important to measure its volume accurately. This is best accomplished with an accurately calibrated 5 ml pipet having delivery time of not less than 15 seconds.)

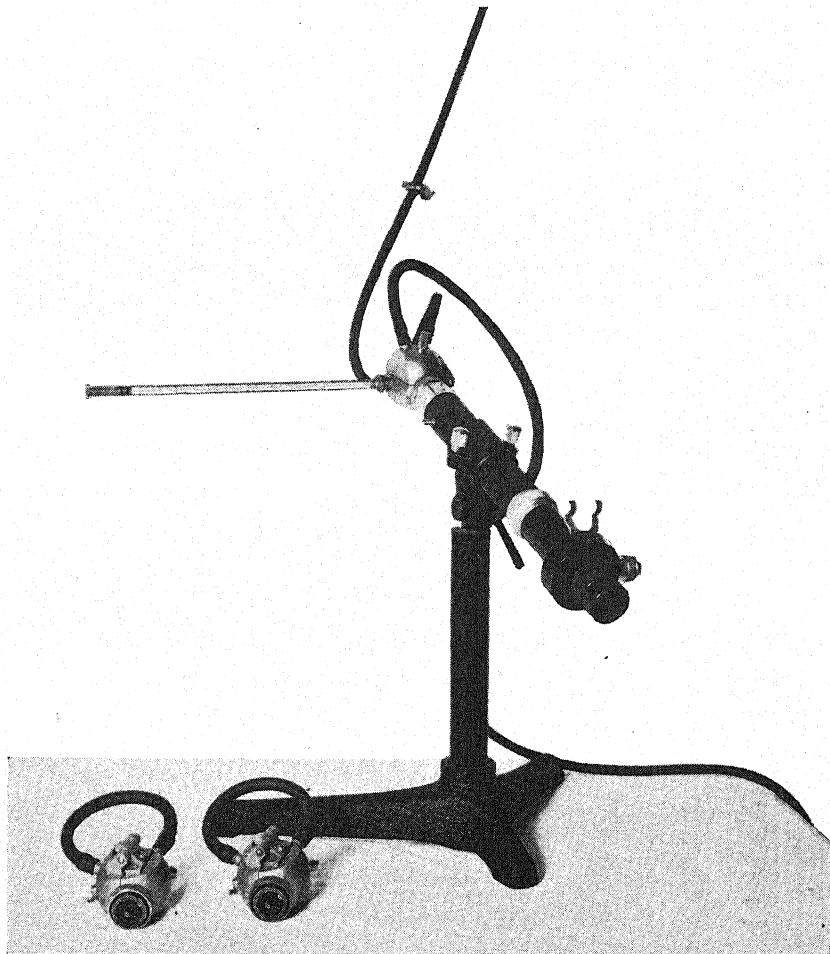


FIG. 45.—DIPPING-TYPE REFRACTOMETER WITH INTERCHANGEABLE DOUBLE PRISM HEADS SUITABLE FOR REFRACTOMETRIC DETERMINATION OF OIL CONTENT OF FLAXSEED.

Grind mixture in mortar vigorously for 3 min., constantly scraping into the bottom particles of meal that are thrown against sides of mortar. Filter mixture into test tube thru a Schleicher and Schull No. 588 folded paper, or other fat-free paper that will yield a clear filtrate. When filtrate has cooled to room temp., determine its refractive index at  $25.0^{\circ}$  to accuracy of  $\pm 0.00002$ . (A dipping-type refractometer equipped with interchangeable, water-jacketed, double prism heads is recommended, Fig. 45.) If reading is made at temp. other than  $25.0^{\circ}$ , make correc-

tion as directed in instructions for preparation of the standard solvent, using temp. coefficient of 0.00042 per 1°. Using Table 1, note percentage of oil corresponding to refractive index of filtrate (*uncorrected value* for oil content).

TABLE 1.—*Conversion table for determining percentage of oil in flaxseed from refractive index of the halowax,  $\alpha$ -bromonaphthalene extract at 25.0°*

$n_D^{25}$	Oil	$n_D^{25}$	Oil	$n_D^{25}$	Oil	$n_D^{25}$	Oil
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>
1.61837	28.0	1.61554	32.5	1.61279	37.0	1.61012	41.5
1.61831	28.1	1.61548	32.6	1.61273	37.1	1.61006	41.6
1.61824	28.2	1.61542	32.7	1.61267	37.2	1.61000	41.7
1.61818	28.3	1.61535	32.8	1.61261	37.3	1.60995	41.8
1.61811	28.4	1.61529	32.9	1.61255	37.4	1.60989	41.9
1.61805	28.5	1.61523	33.0	1.61249	37.5	1.60983	42.0
1.61799	28.6	1.61517	33.1	1.61243	37.6	1.60977	42.1
1.61792	28.7	1.61511	33.2	1.61237	37.7	1.60971	42.2
1.61786	28.8	1.61504	33.3	1.61231	37.8	1.60966	42.3
1.61779	28.9	1.61498	33.4	1.61225	37.9	1.60960	42.4
1.61773	29.0	1.61492	33.5	1.61219	38.0	1.60954	42.5
1.61767	29.1	1.61486	33.6	1.61213	38.1	1.60948	42.6
1.61760	29.2	1.61480	33.7	1.61207	38.2	1.60942	42.7
1.61754	29.3	1.61473	33.8	1.61201	38.3	1.60937	42.8
1.61748	29.4	1.61467	33.9	1.61195	38.4	1.60931	42.9
1.61742	29.5	1.61461	34.0	1.61189	38.5	1.60925	43.0
1.61735	29.6	1.61455	34.1	1.61183	38.6	1.60919	43.1
1.61729	29.7	1.61449	34.2	1.61177	38.7	1.60913	43.2
1.61723	29.8	1.61443	34.3	1.61171	38.8	1.60908	43.3
1.61716	29.9	1.61437	34.4	1.61165	38.9	1.60902	43.4
1.61710	30.0	1.61431	34.5	1.61159	39.0	1.60896	43.5
1.61704	30.1	1.61424	34.6	1.61153	39.1	1.60890	43.6
1.61697	30.2	1.61418	34.7	1.61147	39.2	1.60884	43.7
1.61691	30.3	1.61412	34.8	1.61141	39.3	1.60879	43.8
1.61685	30.4	1.61406	34.9	1.61135	39.4	1.60873	43.9
1.61679	30.5	1.61400	35.0	1.61130	39.5	1.60867	44.0
1.61672	30.6	1.61394	35.1	1.61124	39.6	1.60861	44.1
1.61666	30.7	1.61388	35.2	1.61118	39.7	1.60856	44.2
1.61660	30.8	1.61382	35.3	1.61112	39.8	1.60850	44.3
1.61653	30.9	1.61376	35.4	1.61106	39.9	1.60844	44.4
1.61647	31.0	1.61370	35.5	1.61100	40.0	1.60839	44.5
1.61641	31.1	1.61363	35.6	1.61094	40.1	1.60833	44.6
1.61635	31.2	1.61357	35.7	1.61088	40.2	1.60827	44.7
1.61628	31.3	1.61351	35.8	1.61082	40.3	1.60821	44.8
1.61622	31.4	1.61345	35.9	1.61076	40.4	1.60816	44.9
1.61616	31.5	1.61339	36.0	1.61071	40.5	1.60810	45.0
1.61610	31.6	1.61333	36.1	1.61065	40.6	1.60804	45.1
1.61604	31.7	1.61327	36.2	1.61059	40.7	1.60799	45.2
1.61597	31.8	1.61321	36.3	1.61053	40.8	1.60793	45.3
1.61591	31.9	1.61315	36.4	1.61047	40.9	1.60787	45.4
1.61585	32.0	1.61309	36.5	1.61041	41.0	1.60782	45.5
1.61579	32.1	1.61303	36.6	1.61035	41.1	1.60776	45.6
1.61573	32.2	1.61297	36.7	1.61029	41.2	1.60770	45.7
1.61566	32.3	1.61291	36.8	1.61024	41.3	1.60764	45.8
1.61560	32.4	1.61285	36.9	1.61018	41.4		

Place ca 2 g of the ground sample in fine paper filter in glass funnel and pour over it ca 15 ml of petroleum benzin, collecting clear filtrate in small shallow evaporating dish. Carefully evaporate off the ether on steam bath or hot plate at low heat, and place dish in oven at 105° for 20 min. Cool oil thus prepared to room temp. (If preferred, prepare this sample of oil by pressing small sample of the ground seed in

TABLE 2.—*Corrections\* to be applied to results obtained in analysis of flaxseed for oil content by refractometric method*  
 [Values to be added when  $(n_D^{25}-1.4778)$  is positive, subtracted when  $(n_D^{25}-1.4778)$  is negative]  
 (Corrections in terms of per cent of oil indicated)

$n_D^{25}-1.4778$	28*	30*	31*	32*	33*	34*	35*	36*	37*	38*	39*	40*	41*	42*	43*	44*	45*	46*	47*	48*
0.0001.....	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.03
0.0002.....	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
0.0003.....	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
0.0004.....	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09	0.09	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10
0.0005.....	0.08	0.08	0.09	0.09	0.09	0.10	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.11	0.12	0.12	0.12	0.12	0.12	0.12
0.0006.....	0.09	0.10	0.10	0.11	0.11	0.11	0.12	0.12	0.12	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14
0.0007.....	0.11	0.12	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17
0.0008.....	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.18	0.18	0.18	0.19	0.19	0.20	0.20	0.20	0.20	0.20
0.0009.....	0.14	0.15	0.15	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20	0.21	0.21	0.21	0.22	0.22	0.22	0.22	0.22	0.22
0.0010.....	0.17	0.17	0.17	0.18	0.19	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24	0.24
0.0011.....	0.17	0.17	0.18	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.24	0.25	0.25	0.25	0.25	0.25
0.0012.....	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.25	0.25	0.26	0.26	0.26	0.26	0.26	0.26	0.26
0.0013.....	0.20	0.21	0.22	0.22	0.23	0.24	0.24	0.25	0.25	0.26	0.27	0.27	0.28	0.28	0.29	0.29	0.29	0.29	0.29	0.29
0.0014.....	0.22	0.23	0.24	0.25	0.25	0.26	0.27	0.27	0.28	0.29	0.30	0.31	0.31	0.32	0.32	0.33	0.33	0.33	0.33	0.33
0.0015.....	0.23	0.24	0.25	0.26	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.35	0.36	0.37	0.37	0.37	0.37
0.0016.....	0.24	0.25	0.26	0.27	0.28	0.30	0.31	0.32	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.38	0.39	0.40	0.40	0.40
0.0017.....	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.40	0.41	0.42	0.43	0.43	0.43
0.0018.....	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47	0.48	0.48
0.0019.....	0.29	0.30	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.41	0.42	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.50
0.0020.....	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.42	0.43	0.45	0.46	0.47	0.48	0.50	0.51	0.52	0.53	0.53	0.53
0.0021.....	0.33	0.35	0.36	0.37	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.50	0.51	0.52	0.53	0.55	0.55
0.0022.....	0.34	0.35	0.37	0.38	0.39	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.50	0.51	0.52	0.53	0.55	0.56	0.56
0.0023.....	0.35	0.37	0.38	0.39	0.41	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.51	0.52	0.54	0.55	0.56	0.57	0.59	0.59
0.0024.....	0.37	0.38	0.40	0.41	0.43	0.44	0.46	0.47	0.49	0.51	0.52	0.54	0.55	0.56	0.60	0.61	0.63	0.64	0.66	0.66
0.0025.....	0.38	0.40	0.42	0.43	0.45	0.46	0.48	0.49	0.51	0.53	0.56	0.60	0.61	0.63	0.64	0.68	0.69	0.71	0.72	0.72
0.0026.....	0.40	0.41	0.43	0.45	0.46	0.48	0.50	0.51	0.53	0.56	0.60	0.62	0.64	0.65	0.69	0.70	0.73	0.74	0.75	0.75
0.0027.....	0.41	0.43	0.45	0.46	0.48	0.50	0.52	0.53	0.57	0.58	0.62	0.64	0.66	0.68	0.69	0.71	0.73	0.74	0.76	0.76
0.0028.....	0.43	0.45	0.46	0.48	0.50	0.52	0.55	0.57	0.59	0.61	0.65	0.67	0.69	0.71	0.72	0.74	0.76	0.77	0.79	0.79
0.0029.....	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.59	0.61	0.63	0.67	0.69	0.71	0.73	0.74	0.76	0.78	0.79	0.81	0.81
0.0030.....	0.46	0.48	0.50	0.52	0.54	0.55	0.57	0.61	0.63	0.65	0.67	0.69	0.71	0.73	0.74	0.76	0.78	0.80	0.82	0.82

\* Per cent oil as determined from Table 1.

a laboratory hydraulic press and filtering oil so obtained if it is not entirely clear.) Determine refractive index of the oil at 25.0°. The temp. coefficient for the pure oil is 0.000357 per 1.0°, to be added if temp. at which reading is taken is above 25.0° and subtracted if below that temp.

From the refractive index value of the oil subtract the value 1.47780 (refractive index at 25.0° of composite sample of oil used in obtaining data for Table 1). Using this difference, determine from Table 2, the correction to be applied to the uncorrected value for oil content as determined above. If difference is positive, add correction; if negative, subtract.

#### SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., **15**, 77 (1932); **16**, 77 (1933); Ind. Eng. Chem., **18**, 1350 (1926).
- <sup>2</sup> Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 6th ed., **1**, 313 (1921); J. Soc. Chem. Ind., **26**, 513 (1907).
- <sup>3</sup> Wiley, Principles and Practice of Agricultural Analysis, 2nd ed., **3**, p. 414 (1906-14); Conn. Agr. Exp. Sta. Rpt., 1900, p. 142.
- <sup>4</sup> Ber., **15**, 1031 (1882); J. Am. Chem. Soc., **21**, 991 (1899).
- <sup>5</sup> J. Soc. Chem. Ind., **17**, 1021 (1898).
- <sup>6</sup> U. S. Dept. Agr. Bur. Chem. Bull. **13** (IV), p. 448; Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 6th ed., **1**, 325 (1921); Wiley Principles and Practice of Agricultural Analysis, 2nd ed., **3**, 309 (1906-14).
- <sup>7</sup> J. Assoc. Official Agr. Chem., **19**, 95 (1936).
- <sup>8</sup> Ibid., **6**, 440 (1923).
- <sup>9</sup> Ibid., **19**, 96 (1936).
- <sup>10</sup> Ibid., **13**, 43 (1930).
- <sup>11</sup> Z. Nahr. Genussm., **9**, 65 (1905); J. Assoc. Official Agr. Chem., **13**, 44 (1930); Analyst, **36**, 333 (1911).
- <sup>12</sup> J. Assoc. Official Agr. Chem., **12**, 44-46 (1929).
- <sup>13</sup> Ibid., **10**, 35 (1927).
- <sup>14</sup> U. S. Dept. Agr., Bur. Animal Ind. Circ., **212**.
- <sup>15</sup> Chem. Ztg., **37**, 1001 (1913).
- <sup>16</sup> J. Ind. Eng. Chem., **11**, 161 (1919); J. Assoc. Official Agr. Chem., **8**, 85 (1924); **8**, 484 (1925); **9**, 45 (1926).
- <sup>17</sup> Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 6th ed., **1**, 350 (1921).
- <sup>18</sup> J. pharm. chim., 6th ser., **6**, 390 (1897); Abs. Analyst, **22**, 326 (1897); Allen, Commercial Organic Analysis, 4th ed., **2**, 135 (1909); Conn. Agr. Exp. Sta. Rpt., 1900 (II), p. 143.
- <sup>19</sup> Allen, Commercial Organic Analysis, 4th ed., **2**, 135 (1909-14).
- <sup>20</sup> Abs. J. Soc. Chem. Ind., **18**, 711 (1899).
- <sup>21</sup> Compt. rend., **73**, 1330 (1871); Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 6th ed., **2**, 316 (1922).
- <sup>22</sup> J. Am. Chem. Soc., **15**, 110 (1893).
- <sup>23</sup> J. Assoc. Official Agr. Chem., **12**, 46 (1929).
- <sup>24</sup> Ibid., **19**, 496 (1936); **20**, 74 (1937); **21**, 87 (1938).
- <sup>25</sup> J. Soc. Chem. Ind., **12**, 67 (1893); **13**, 69 (1894); J. Assoc. Official Agr. Chem., **6**, 441 (1923).
- <sup>26</sup> U. S. Dept. Agr. Bur. Animal Ind. Circ., **132**; J. Assoc. Official Agr. Chem., **19**, 97 (1936).
- <sup>27</sup> U. S. Dept. Agr. Bur. Agr. Economics, S.R.A. 133, August, 1932, revised July 5, 1939; J. Assoc. Official Agr. Chem., **17**, 70 (1934).
- <sup>28</sup> U. S. Dept. Agr. Bull. **1471** (1927); J. Assoc. Official Agr. Chem., **20**, 421 (1937).

#### SPECIAL REFERENCES

- ELSDON, Edible Fats and Oils, E. Benn, Ltd., London (1926).  
 HILDITCH, Fats and Waxes, Bailliere, Tindall & Cox, London (1927); J. Soc. Chem. Ind., **49**, 210 (1930); **54**, 139, 163, 184 (1935).  
 ALSBERG and TAYLOR, The Fats and Oils: A General Review, Food Research Institute, Stanford University, California.  
 SNODGRASS, Copra and Coconut Oil, Food Research Institute, Stanford University, California; Margarine as a Butter Substitute (1930).

BOLTON, Oils, Fats and Fatty Foods, P. Blakiston's Sons Co. Philadelphia (1928).  
GRÜN, Analyse der Fette und Wachse, Vol. 1 (1925); Vol. 2 (1929), J. Springer, Berlin.

ELLIS, Hydrogenation of Organic Substances, 3rd Ed., D. Van Nostrand Co., New York (1930).

JAMIESON, Vegetable Fats and Oils, Chemical Catalog Co., New York (1932).

ZAPOLEON, Inedible Animal Fats in the United States (1929).

WEBER and ALSBERG, The American Vegetable Shortening Industry, Food Research Institute, Stanford University, California (1934).

DRUMMUND, J. Soc. Chem. Ind., 45, 994 (1926).

ANDREWS, Colour Trades J., 72, 1421, 1581 (1927).

UBBELOHDE's Handbuch der Öle und Fette, vol. 1, 2nd ed. H. Heller (1929); Vol. 2 (1932); Vol. 3 (1929); Vol. 4, Ubbelohde, Goldschmidt and Hartmann (1926). S. Hirzel, Leipzig.

HEFTER, Chemie und Technologie der Fette und Fett Produkte, 2nd. Ed. H. SCHÖNFELD and collaborators, Vol. 1 (1936); Vol. 2 (1937). JULIUS SPRINGER, Wien.

DEAN, Utilization of Fats. A Theoretical and Technical Treatise on Composition, extraction, analysis, and application of fats. Chemical Publishing Co., New York (1938).



## XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

### SALICYLIC ACID

1

#### PREPARATION OF SAMPLE—OFFICIAL

(a) *Non-alcoholic liquids*.—Many liquids may be extracted directly as described under 2 or 4 without further treatment. If troublesome emulsions form during extraction, pipet 100 ml into 250 ml volumetric flask, and add ca 5 g of NaCl, shaking until dissolved. Make up to mark with alcohol, shake vigorously, allow mixture to stand for 10 min. with occasional shaking, filter, and treat aliquot of filtrate as directed under (b).

(b) *Alcoholic liquids*.—Make 200 ml of sample alkaline with ca 10% NaOH soln, using litmus paper as indicator, and evaporate on steam bath to ca  $\frac{1}{2}$  its original volume. Dilute to original volume with H<sub>2</sub>O and filter if necessary.

(c) *Solid or semi-solid substances*.—Grind sample and mix thoroly. Transfer convenient quantity (50–200 g according to consistency of sample) to 500 ml volumetric flask, add sufficient H<sub>2</sub>O to make a volume of ca 400 ml, and shake until mixture becomes uniform. Add 2–5 g of CaCl<sub>2</sub> and shake until dissolved; render distinctly alkaline with ca 10% NaOH soln, using litmus paper as indicator; fill to mark with H<sub>2</sub>O, shake thoroly, allow to stand for at least 2 hours, shaking frequently, and filter.

#### QUALITATIVE TESTS

2

#### Ferric Chloride Test—Official

Introduce 50 ml of sample or equivalent quantity of aqueous extract, prepared as directed under 1, into separatory funnel; add 1/10 its volume of HCl (1+3) and extract with 50 ml of ether. If mixture emulsifies, add 10–15 ml of petroleum benzin (b.p. below 60°) and shake. If this treatment fails to break the emulsion, whirl mixture in centrifuge, or allow it to stand until a considerable portion of aqueous layer has separated; run off latter, shake vigorously, and again allow to separate. Wash ether layer with two 5 ml portions of H<sub>2</sub>O, evaporate greater portion of the ether in porcelain dish on steam bath, allow remainder to evaporate spontaneously, and add a drop of 0.5% neutral FeCl<sub>3</sub> soln. A violet color indicates salicylic acid.

If coloring matter or other interfering substance is present in residue after evaporation of ether, purify the salicylic acid by one of following methods:

(a) Dissolve original residue from ether extract, obtained as directed above, in ca 25 ml of ether; transfer this soln to separatory funnel and shake with equal volume of H<sub>2</sub>O made distinctly alkaline with several drops of 10% NH<sub>4</sub>OH. Allow to separate, filter aqueous layer thru wet filter into porcelain dish, evaporate almost to dryness, and test residue as directed previously.

(b) Dry original residue from ether extract, obtained as directed above, in desiccator over H<sub>2</sub>SO<sub>4</sub> and extract with several 10 ml portions of CS<sub>2</sub> or petroleum benzin (b.p. below 60°), rubbing contents of dish with glass rod and filtering the successive portions of the solvent thru dry paper into a second porcelain dish. Evaporate the greater portion of solvent on steam bath, allow remainder to evaporate spontaneously, and test residue as directed previously.

(c) By means of a few ml of ether, transfer original residue from the ether extract, obtained as directed above, to small porcelain crucible, and allow solvent to evaporate spontaneously. Cut a hole in an asbestos board sufficiently large to admit ca  $\frac{2}{3}$  of crucible, cover with small, round-bottomed flask filled with cold H<sub>2</sub>O, and heat

over small Bunsen flame until any salicylic acid present has sublimed and condensed upon bottom of flask. Test sublimate as directed previously.

3

*Jorissen Test*<sup>1</sup>—Official

Dissolve residue from the ether extract, 2, or, if impurities are present, the purified material obtained as directed under 2(a), (b), or (c), in a little hot H<sub>2</sub>O. Cool 10 ml of soln in test tube; add 4 or 5 drops of 10% KNO<sub>2</sub> soln, 4 or 5 drops of acetic acid (ca 50% soln), and 1 drop of 1% CuSO<sub>4</sub> soln; mix thoroly, boil the liquid for half a minute, and allow to stand for 2 min. In presence of salicylic acid a Bordeaux-red color develops.

## QUANTITATIVE METHOD—OFFICIAL

4

## EXTRACTION

Transfer to a separatory funnel 100 ml of sample, or that quantity of a soln prepared as directed under 1 which represents not less than 20 g of original material. If alkaline, neutralize to litmus with HCl (1+3) and add excess of HCl equivalent to 2 ml of acid for each 100 ml of soln. Extract with 4 separate portions of ether, using for each extraction a volume of ether equivalent to  $\frac{1}{2}$  the volume of aqueous layer. If emulsion forms on shaking, this may usually be broken by adding a little ( $\frac{1}{2}$  volume of ether layer) petroleum benzin (b.p. below 60°) and shaking again, or by centrifuging. If small quantity of emulsion still persists, allow it to remain with aqueous layer, where frequently it is broken during next extraction. If an emulsion remains after fourth extraction, separate it from the clear ether and the clear aqueous layer and extract it separately with 2 or 3 small portions of ether. Combine ether extracts, wash with volume of H<sub>2</sub>O equal to 1/10 of total volume of ether extracts, allow layers to separate, and reject aqueous layer. Wash in this way until aqueous layer after separation yields yellow color upon addition of methyl orange soln and 2 drops of 0.1 N NaOH. Distil slowly greater part of ether, transfer remainder to porcelain dish, and allow it to evaporate spontaneously. If no interfering substances are present, proceed as directed under 5; if interfering substances are present, purify residue by one of following methods:

(a) Thoroly dry residue in vacuo over H<sub>2</sub>SO<sub>4</sub>. Extract it 10 times with 10–15 ml portions each of CS<sub>2</sub> or petroleum benzin (b.p. below 60°), rubbing contents of dish with glass rod, and filter successive portions of solvent thru dry filter into porcelain dish. Test extracted residue with a drop of 2% ferric alum soln, and if it gives reaction for salicylic acid, dissolve it in H<sub>2</sub>O; acidify soln with HCl (1+3), extract with ether, evaporate, extract dry residue thus obtained with CS<sub>2</sub> or petroleum benzin, and add to extract first obtained. Distil greater portion of the CS<sub>2</sub> or petroleum benzin and allow remainder to evaporate spontaneously. Proceed as directed under 5.

(b) Dissolve residue in 40–50 ml of ether. Transfer ether soln to a separatory funnel and extract with 3 successive 15 ml portions of 1% NH<sub>4</sub>OH. (If fat is known to be present in original ether extract, extract latter directly with 4 portions of the NH<sub>4</sub>OH instead of 3.) Combine alkaline aqueous extracts, acidify, again extract with ether, and wash combined ether extracts as directed previously. Slowly distil greater portion of the ether, allow remainder to evaporate spontaneously, and proceed as directed under 5.

5

## DETERMINATION

Dissolve residue, 4, in small quantity of hot H<sub>2</sub>O, and after cooling dilute to definite volume (usually 50 or 100 ml). If soln is not clear, filter thru dry filter. Dilute aliquots of the soln and treat with 0.5% FeCl<sub>3</sub> soln or 2% ferric alum soln until maximum color is developed. Generally a few drops will suffice.

(The ferric alum soln should be boiled until precipitate appears, allowed to settle, and filtered. The acidity of the soln is slightly increased in this manner, but the soln remains clear for a considerable time, and the turbidity caused by its dilution with  $H_2O$  is much less and does not appear so soon as when the unboiled soln is used. This turbidity interferes with the exact matching of the color.)

Compare colors developed with color obtained when a standard salicylic acid soln (containing 1 mg of salicylic acid in 50 ml) is similarly treated, using Nessler tubes or a colorimeter. In either case, and especially with  $FeCl_3$ , avoid excess of the reagent, altho an excess of 0.5 ml of 2% ferric alum soln may be added to 50 ml of the comparison soln of salicylic acid without vitiating the results.

## BENZOIC ACID

### QUALITATIVE TESTS

6

#### *I. Preliminary Test—Official*

Extract benzoic acid as directed under 2 or 4. If benzoic acid is present in considerable quantity, it will crystallize from the ether in shining leaflets having characteristic odor on warming. Dissolve crystalline deposit in hot  $H_2O$ , divide into 2 portions, and test as directed in 7 or 8. The deposit may also be purified as directed under 2(c) and the melting point determined.

7

#### *II. Ferric Chloride Test—Official*

Make soln, 6, alkaline with a few drops of  $NH_4OH$ , expel excess of  $NH_3$  by evaporation, dissolve residue in few ml of hot  $H_2O$ , filter if necessary, and add a few drops of neutral 0.5%  $FeCl_3$  soln. A salmon-colored precipitate of ferric benzoate indicates presence of benzoic acid.

8

#### *III. Modified Mohler Test<sup>2</sup>—Official*

(Presence of phenolphthalein interferes with this test.)

Add to the  $H_2O$  soln, 6, 1 or 2 drops of ca 10%  $NaOH$  soln and evaporate to dryness. To residue add 5–10 drops of  $H_2SO_4$  and a small crystal of  $KNO_3$ . Heat for 10 min. in bath (glycerol) at 120–130° (temp. must not exceed 130°). After cooling, add 1 ml of  $H_2O$  and make distinctly ammoniacal. Boil soln to decompose any  $NH_4NO_2$  that might have been formed. Cool, and add a drop of fresh, colorless  $(NH_4)_2S$  soln, but do not allow layers to mix. A red-brown ring indicates benzoic acid. On mixing the color diffuses thruout the liquid, and on heating finally changes to greenish yellow. This change differentiates benzoic acid from salicylic acid or cinnamic acid. The salicylic and cinnamic acids form colored compounds that are not destroyed by heating.

### QUANTITATIVE METHODS—OFFICIAL

#### PREPARATION OF SAMPLE

9

#### *General Method*

Mix sample thoroly, grinding if solid or semi-solid. Transfer 150 ml or 150 g to 500 ml volumetric flask, add enough pulverized  $NaCl$  to saturate the  $H_2O$  in sample, make alkaline to litmus paper with 10%  $NaOH$  soln or with milk of lime (1 part powdered recently slaked lime suspended in 3 parts of  $H_2O$ ), and dilute to mark with saturated  $NaCl$  soln. Shake thoroly, allow to stand for at least 2 hours, with frequent shaking, and filter.

If sample contains large quantities of fat, portions of which may contaminate a filtrate, add a few ml of the NaOH soln to filtrate and extract with ether before proceeding as directed under 11. If alcohol is present, proceed as directed under 10(c). If sample contains large quantities of matter precipitable by salt soln, proceed as directed under 10(d).

10

*Special Methods*

(a) *Ketchup*.—To 150 g of sample add 15 g of pulverized NaCl, and transfer mixture to 500 ml volumetric flask, rinsing with ca 150 ml of saturated NaCl soln. Make slightly alkaline to litmus paper with 10% NaOH soln and fill to mark with saturated NaCl soln. Allow to stand at least 2 hours, shaking frequently. Squeeze thru heavy muslin bag and filter.

(b) *Jellies, jams, preserves, and marmalades*.—Digest 150 g of sample in ca 300 ml of saturated NaCl soln. Add 15 g of pulverized NaCl. Make alkaline to litmus paper with milk of lime. Transfer to 500 ml volumetric flask and dilute to mark with saturated NaCl soln. Allow to stand at least 2 hours, shaking frequently; centrifuge if necessary, and filter.

(c) *Cider containing alcohol, and similar products*.—Make 250 ml of sample alkaline to litmus paper with 10% NaOH soln and evaporate on steam bath to ca 100 ml. Transfer sample to 250 ml volumetric flask, add 30 g of pulverized NaCl, and shake until dissolved. Dilute to original volume, 250 ml, with saturated NaCl soln; allow to stand at least 2 hours, shaking frequently, and filter.

(d) *Salted or dried fish*.—Wash 50 g of ground sample into 500 ml volumetric flask with H<sub>2</sub>O. Make slightly alkaline to litmus paper with 10% NaOH soln and dilute to mark with H<sub>2</sub>O. Allow to stand at least 2 hours, shaking frequently, and filter. Pipet as large a measured portion of filtrate as possible (at least 300 ml) into second 500 ml flask, adding 30 g of the pulverized NaCl for each 100 ml of soln. Shake until the NaCl has dissolved and dilute to mark with saturated NaCl soln. Mix thoroly and filter off precipitated protein and other extraneous matter.

11

## DETERMINATION

Pipet a convenient portion (100–200 ml) of filtrate, 9 or 10, into a separatory funnel. Neutralize soln to litmus paper with HCl (1+3) and add excess of 5 ml of the same acid. In the case of salted fish a precipitation of protein matter usually occurs on acidifying, but the precipitate does not interfere with the extraction. Extract carefully with CHCl<sub>3</sub>, using successively portions of 70, 50, 40, and 30 ml. To avoid formation of an emulsion, shake cautiously each time, using rotary motion. The CHCl<sub>3</sub> layer usually separates readily after standing a few minutes. If an emulsion forms, break it by stirring CHCl<sub>3</sub> layer with glass rod, by drawing it off into second funnel and giving one or two sharp shakes from one end of funnel to other, or by centrifuging for a few minutes. As this is a progressive extraction, draw off carefully as much of the clear CHCl<sub>3</sub> soln as possible after each extraction, but do not draw off any of emulsion with the CHCl<sub>3</sub> layer. If this precaution is taken, the CHCl<sub>3</sub> extract need not be washed.

Transfer combined CHCl<sub>3</sub> extracts to porcelain evaporating dish, rinse container several times with a few ml of CHCl<sub>3</sub>, and evaporate to dryness at room temp. in current of dry air.

The extract may also be transferred from the separatory funnel to a 300 ml Erlenmeyer flask, and the separatory funnel rinsed 3 times with 5–10 ml portions of CHCl<sub>3</sub>. Distil very slowly at low temp. to ca  $\frac{1}{2}$  original volume. Transfer residue to porcelain evaporating dish, rinsing flask 3 times with 5–10 ml portions of CHCl<sub>3</sub>, and evaporate to dryness at room temp. in current of dry air.

Dry residue overnight (or until no odor of acetic acid can be detected if the product is a ketchup) in desiccator containing  $\text{H}_2\text{SO}_4$ . Dissolve residue of benzoic acid in 30–50 ml of alcohol neutral to phenolphthalein; add ca  $\frac{1}{4}$  this volume of  $\text{H}_2\text{O}$  and 1 or 2 drops of phenolphthalein indicator, II, 10(d); and titrate with 0.05  $N$  NaOH. 1 ml of 0.05  $N$  NaOH = 0.0072 g of anhydrous Na benzoate.

### SACCHARIN

#### 12

##### PREPARATION OF SAMPLE—OFFICIAL

(a) *Fruit juices and sirups*.—Transfer 100–200 g of sample to 250 ml volumetric flask by means of a little  $\text{H}_2\text{O}$  and dilute to ca 200 ml with  $\text{H}_2\text{O}$ . Add 5 ml of glacial acetic acid and mix. Add slight excess of 20% neutral Pb acetate soln, mix thoroly, dilute to mark with  $\text{H}_2\text{O}$ , again mix thoroly, and filter.

(b) *Alcoholic liquids*.—Heat 100–200 ml of liquid on steam bath to remove alcohol (usually done by evaporating to  $\frac{1}{2}$  original volume). With heavy sirups, dilute liquid with equal volume of  $\text{H}_2\text{O}$  before beginning evaporation. After alcohol has been removed, transfer liquid to 250 ml volumetric flask and proceed as directed under (a).

(c) *Solid or semi-solid preparations*.—Transfer 50–75 g of sample to 250 ml volumetric flask by means of a little hot  $\text{H}_2\text{O}$  and add sufficient boiling  $\text{H}_2\text{O}$  to make volume ca 200 ml. Allow mixture to stand for 2 hours, shaking occasionally. Add 5 ml of glacial acetic acid, mix thoroly, add slight excess of 20% neutral Pb acetate soln, dilute to mark with cold  $\text{H}_2\text{O}$ , mix, allow to stand for 20 min., and filter.

#### 13

##### QUALITATIVE TEST—OFFICIAL

(a) Acidify 50 ml of non-alcoholic liquid foods or the aqueous extract of 50 g of a solid or semi-solid product, prepared as directed under 12, with HCl and extract 3 times with 25 ml portions of ether. Wash combined ether extracts once with 5 ml of  $\text{H}_2\text{O}$ , transfer to small beaker or evaporating dish, allow ether to evaporate spontaneously, and taste residue. (The presence of as little as 20 mg of saccharin per liter or kg of original sample can usually be detected by its sweet taste.) Confirm by heating with NaOH and detecting the salicylic acid formed thereby as directed under (b).

(b) Acidify with HCl 50 ml of a non-alcoholic liquid food, or equivalent quantity of aqueous extract, 12, and extract with 3 portions of ether as directed under (a). Dissolve residue remaining after evaporation of the ether in a little hot  $\text{H}_2\text{O}$  and test small portion of the soln for salicylic acid as directed under 2 or 3. Dilute remainder of soln to ca 10 ml and add 2 ml of  $\text{H}_2\text{SO}_4$  (1+3). Heat to boiling and add slight excess of 5%  $\text{KMnO}_4$  soln dropwise; partly cool soln, dissolve ca 1 g of NaOH in it, and filter mixture into an Ag dish (Ag crucible lids are well adapted to purpose). Evaporate to dryness and heat 20 min. at 210–215°. Dissolve residue in  $\text{H}_2\text{O}$ , acidify with HCl, and test ether extract for salicylic acid as directed under 2 or 3. By this method all so-called “false saccharin” and any salicylic acid naturally present (also added salicylic acid when not present in too large a quantity) are destroyed, whereas 5 mg of saccharin per liter is detected with certainty.

### QUANTITATIVE METHODS

#### 14

##### I. General Method—Official

Transfer 150 ml of filtrate, 12, to a separatory funnel, add 15 ml of HCl, and extract 3 times with 80 ml portions of ether, shaking separatory funnel 2 min. each time. Wash combined ether extracts once with 5 ml of  $\text{H}_2\text{O}$ , remove ether by distillation,

and transfer residue to Pt crucible by means of a little ether; or, if substances difficultly soluble in ether are present, use alternately small portions of  $\text{H}_2\text{O}$  and ether. Evaporate the ether on steam bath, add to residue 2–3 ml (or enough to make mixture strongly alkaline) of 10%  $\text{Na}_2\text{CO}_3$  soln, rotate so that all saccharin is brought in contact with the soln, and evaporate to dryness on steam bath. To dry residue in crucible add 4 g of a mixture of equal parts of anhydrous  $\text{Na}_2\text{CO}_3$  and  $\text{K}_2\text{CO}_3$ . Heat gently at first and then to complete fusion for 30 min. over alcohol or other S-free flame. (The fusion may be conducted by closely fitting crucible into hole cut into piece of heavy asbestos board so that  $\frac{1}{2}$  of crucible projects above the asbestos, and heating lower portion of crucible by means of large Bunsen, Meker, or similar burner.) Cool, dissolve melt in  $\text{H}_2\text{O}$ , add ca 5 ml of Br water, acidify with  $\text{HCl}$ , filter, wash paper with a little  $\text{H}_2\text{O}$ , dilute filtrate and washings to ca 200 ml, heat to boiling, and slowly add excess of  $\text{BaCl}_2$  soln (ca 10%). Allow mixture to stand overnight, collect the  $\text{BaSO}_4$  on filter or on Gooch crucible, wash until free from chlorides, dry, ignite, cool, and weigh. Correct result thus obtained for any S present in fusion mixture as found by blank determination. Calculate equivalent quantity of saccharin by multiplying corrected weight of  $\text{BaSO}_4$  by 0.7844.

(Instead of the mixed Na and K carbonates, 3–4 g of  $\text{Na}_2\text{O}_2$  may be used for the fusion. In this case a Ni crucible must be used, and time of fusion may be reduced to 5 min. The separation of a little  $\text{PbCl}_2$  during the extractions does not interfere with accuracy of method.)

15

### II. Special Method—Official, First Action

*Non-alcoholic beverages.*<sup>3</sup>—Add 2 ml of  $\text{HCl}$  to 50 ml of the drink contained in a separatory funnel. Extract with two successive 50 ml portions of ether. Filter ether extractions thru cotton, and wash combined filtrates with ca 5 ml of  $\text{H}_2\text{O}$  to which has been added 1 drop of  $\text{HCl}$ .

Separate ethereal layer and evaporate to dryness on water bath. Add to residue 5 ml of ammonia-free  $\text{H}_2\text{O}$  and 6 ml of  $\text{HCl}$  and evaporate soln to ca 1 ml on hot plate with constant stirring. Again add 5 ml of ammonia-free  $\text{H}_2\text{O}$  and 6 ml of  $\text{HCl}$  and evaporate to ca 1 ml. Dilute to 50 ml with ammonia-free  $\text{H}_2\text{O}$  and dilute 2 ml of this soln to 25 ml with ammonia-free  $\text{H}_2\text{O}$ . Add 1 ml of Nessler's reagent, XXXVII, 10(a), and compare with  $\text{NH}_4\text{Cl}$  standards in usual manner; 0.2923 g of dry  $\text{NH}_4\text{Cl}$  = 1 g of saccharin, insoluble form, and = 1.317 g of the sodium salt of the Pharmacopoeia crystallizing with 2 molecules of  $\text{H}_2\text{O}$  of hydration. For convenience prepare  $\text{NH}_4\text{Cl}$  standard equivalent to 200 p.p.m. of the insoluble form of saccharin.

## BORIC ACID AND BORATES

16

### QUALITATIVE TEST<sup>4</sup>—OFFICIAL

*Preliminary test.*—Acidify sample with  $\text{HCl}$  in proportion of 7 ml of acid to each 100 ml of sample. In case of solid or pasty samples heat with enough  $\text{H}_2\text{O}$  to make sufficiently fluid before acidifying. Immerse strip of turmeric paper in the acidified liquid, and allow the paper to dry spontaneously. If borax or  $\text{H}_3\text{BO}_3$  is present, the paper will acquire a characteristic red color, changed by  $\text{NH}_4\text{OH}$  to dark blue-green, but restored by acid.

*Confirmatory test.*—Make ca 25 g of sample decidedly alkaline with lime  $\text{H}_2\text{O}$  or milk of lime and evaporate to dryness on steam bath. Ignite dry residue at low red heat until organic matter is thoroly charred. Cool, digest with ca 15 ml of  $\text{H}_2\text{O}$ , and add  $\text{HCl}$  dropwise until soln is distinctly acid. Immerse piece of turmeric paper in soln and allow to dry without heat. In presence of borax or  $\text{H}_3\text{BO}_3$ , the color change will be same as described under preliminary test.

17

QUANTITATIVE METHOD<sup>1</sup>—OFFICIAL

Make 10–100 g of sample (depending upon nature of material and quantity of  $\text{H}_3\text{BO}_3$  present) distinctly alkaline with NaOH soln and evaporate to dryness in Pt dish. Ignite residue until organic matter is thoroly charred, avoiding intense red heat; cool, digest with ca 20 ml of hot  $\text{H}_2\text{O}$  and add HCl dropwise until reaction is distinctly acid. Filter into 100 ml volumetric flask and wash with a little hot  $\text{H}_2\text{O}$ . (Volume of filtrate should not exceed 50–60 ml.) Return filter containing any unoxidized C to Pt dish, make alkaline by wetting thoroly with lime  $\text{H}_2\text{O}$ , dry on steam bath, and ignite to white ash. Dissolve ash in a few ml of HCl (1+3) and add to liquid in 100 ml flask, rinsing dish with a few ml of  $\text{H}_2\text{O}$ . To combined solns, add 0.5 g of  $\text{CaCl}_2$  and a few drops of phenolphthalein indicator, then 10% NaOH soln until a permanent light pink color is produced. Finally dilute to mark with lime  $\text{H}_2\text{O}$ , mix, and filter thru dry filter. To 50 ml of filtrate add 1 N  $\text{H}_2\text{SO}_4$ , II, 19(b), until pink color disappears, then add methyl orange indicator, VI, 3(f), and continue addition of the acid until yellow color is changed to pink. Boil ca 1 min. to expel  $\text{CO}_2$ . Cool, and carefully add 0.2 N NaOH until liquid assumes yellow tinge, avoiding excess of the alkali. (All the boric acid is now in free state with no uncombined  $\text{H}_2\text{SO}_4$  present.) Add 1–2 g of neutral mannitol and a few drops of phenolphthalein indicator, note buret reading, and again titrate soln with the standard NaOH until pink color develops. Add a little more mannitol, and if pink color disappears continue addition of the standard alkali until pink color reappears. Repeat alternate addition of mannitol and standard alkali until a permanent end point is reached. A volume of glycerol neutral to phenolphthalein equal to volume of soln to be titrated may be substituted for the mannitol. 1 ml of 0.2 N NaOH soln = 0.0124 g of boric acid.

## FORMALDEHYDE

18

## PREPARATION OF SAMPLE—OFFICIAL

If sample is solid or semi-solid, macerate 200–300 g with ca 100 ml of  $\text{H}_2\text{O}$  in mortar. Transfer to short-necked, 500–800 ml Cu or glass distillation flask, make distinctly acid with  $\text{H}_3\text{PO}_4$ , connect with condenser, and distil 40–50 ml. With highly colored liquids, make ca 200 ml distinctly acid with  $\text{H}_3\text{PO}_4$  and distil as directed previously.

## QUALITATIVE TESTS

19

*I. Phenylhydrazin Hydrochloride Test<sup>6</sup>—Official*

(Gives reliable reactions for HCHO in solns varying from 1 part in 50,000 to 1 part in 150,000. Neither acetaldehyde nor benzaldehyde interferes with reaction.)

With milk and other liquids, shake with an equal volume of alcohol, filter and use filtrate. With meats and fats, extract the HCHO with alcohol and use filtrate. With fat, heat mixture above melting point of the fat to insure thoro extraction.

Mix 5 ml of distillate obtained under 18, or of an alcoholic soln or extract, obtained as directed previously, with 0.03 g of phenylhydrazin hydrochloride and 4 or 5 drops of a 1%  $\text{FeCl}_3$  soln. Add 1–2 ml of  $\text{H}_2\text{SO}_4$  slowly and with agitation, keeping the liquid in bath of cold  $\text{H}_2\text{O}$  to prevent heating. Dissolve precipitate by addition of either  $\text{H}_2\text{SO}_4$  (keeping mixture cool) or alcohol. In presence of HCHO a red color develops.

20

*II. Hehner Test<sup>7</sup>—Official*

Mix in test tube ca 5 ml of distillate obtained under 18 with an equal volume of pure milk or with 1–2% soln of egg albumin, and underlay with commercial  $\text{H}_2\text{SO}_4$ .

without mixing. A violet or blue color at junction of the two liquids indicates HCHO. This color is given only in presence of trace of  $\text{FeCl}_3$  or other oxidizing agent. If only pure acid is available, add a few drops of  $\text{FeCl}_3$  soln to acid before making test. Milk may be treated directly by this method, and it gives positive tests in presence of one or more parts of HCHO per 10,000. Other articles of food rich in proteins, for example, egg albumin, give the reaction in the presence of  $\text{H}_2\text{O}$  without addition of milk.

21

### III. Leach Test—Official

Mix in porcelain casserole ca 5 ml of distillate, 18, with an equal volume of pure milk and add ca 10 ml of  $\text{HCl}$  soln containing 2 ml of 10%  $\text{FeCl}_3$  soln to each liter of acid. Heat to  $80\text{--}90^\circ$  directly over gas flame, rotating casserole to break up the curd. A violet coloration indicates HCHO.

### 22 IV. Phenylhydrazin Hydrochloride and Sodium Nitroprusside Test<sup>s</sup>—Official

(Applicable directly to liquid foods, to an aqueous or alcoholic extract of solid foods, or to distillate prepared as directed under 18.)

In the case of milk, apply method directly. With meat, comminute sample, extract with 2 volumes of hot  $\text{H}_2\text{O}$ , and use expressed liquid for test. Heat ca 10 g of fats above their melting point with 20 ml of alcohol, shake thoroly, cool, filter thru moistened filter, and use filtrate for test.

Dissolve a lump of phenylhydrazin hydrochloride about size of a pea in 3–5 ml of liquid to be tested, and add 2–4 drops (not more) of 5–10%  $\text{Na}$  nitroprusside soln and 8–12 drops of an approximately 10%  $\text{NaOH}$  soln. If HCHO is present, a green or blue color, depending upon quantity, develops. When HCHO is present to extent of more than 1 part in 70,000–80,000 in soln tested, a distinct green or bluish green coloration is obtained. In more dilute solns the green tint becomes less marked, and a yellow tinge tending toward greenish brown develops. With this test acetaldehyde and benzaldehyde give a color varying, according to strength of the soln, from red to brown. Therefore, a reaction may be obtained with these aldehydes similar to that obtained with HCHO in solns more dilute than 1 part in 70,000. The presence of acetaldehyde or benzaldehyde together with HCHO gives a yellowish or yellowish green tinge. The reaction for HCHO, therefore, may be masked by presence of other aldehydes, but it is characteristic when a clear green color is obtained.

23

### V. Phenylhydrazin Hydrochloride and Potassium Ferricyanide Test<sup>s</sup>—Official

(Not applicable in presence of coloring matter of blood.)

Proceed as directed under 22, substituting a soln of  $\text{K}_3\text{Fe}(\text{CN})_6$  for the  $\text{Na}$  nitroprusside. HCHO gives a red color. Alcoholic extracts from foods must be diluted with  $\text{H}_2\text{O}$  to prevent precipitation of  $\text{K}_3\text{Fe}(\text{CN})_6$ .

24

### VI. Phenylhydrazin Hydrochloride and Ferric Chloride Test<sup>s</sup>—Official

Treat 15 ml of milk or other liquid food, or of distillate prepared as directed under 18, with 1 ml of 1% phenylhydrazin hydrochloride soln, then with a few drops of 1%  $\text{FeCl}_3$  soln, and finally with  $\text{HCl}$ . The presence of HCHO is indicated by the formation of red color, which changes after some time to orange yellow. Milk may be examined directly by this method, but more delicate tests may be obtained from distillate from milk or from milk serum. Acetaldehyde or benzaldehyde does not interfere with the reaction



25

*VII. Phloroglucinol Test<sup>9</sup>—Official*

To 10 ml of milk or other liquid food under examination in test tube add, by means of pipet, 2 ml of phloroglucinol reagent (1 g of phloroglucinol, 20 g of NaOH, and H<sub>2</sub>O to make 100 ml), placing end of pipet on bottom of tube in such a manner that the reagent will form a separate layer. If HCHO is present, a bright red coloration (not purple) forms at zone of contact.

(This soln gives yellow color in presence of some aldehydes, and if it is used for detection of aldehyde formed by oxidation of methyl alcohol after the destruction of acetaldehyde with H<sub>2</sub>O<sub>2</sub> soln, orange yellow color will slowly appear when insufficient quantity of H<sub>2</sub>O<sub>2</sub> soln has been used. On the other hand, if excess of H<sub>2</sub>O<sub>2</sub> soln is not fully destroyed before use of this reagent, a purple color develops slowly. The clear red color given by this reagent forms quickly, and in presence of but a small quantity of HCHO it fades rapidly.)

**SOLUBLE FLUORIDES**

26

**QUALITATIVE TESTS<sup>10</sup>—OFFICIAL***I. Not Applicable in Presence of Silicates*

After thoroly mixing sample transfer to beaker 150 ml, or an equivalent quantity of aqueous extract in the case of solid foods, and boil, adding 5 ml of 10% K<sub>2</sub>SO<sub>4</sub> soln and 10 ml of 10% Ba acetate soln. Collect precipitate in compact mass (centrifuge may be used advantageously) and wash upon small filter. Transfer to Pt crucible and ignite.

Dip a carefully cleaned glass plate, while hot, in mixture of equal parts of carnauba wax and paraffin and allow to cool. Make a distinctive mark thru the wax with sharp instrument, taking care not to scratch surface of glass.

Add a few drops of H<sub>2</sub>SO<sub>4</sub> to residue in crucible and cover crucible with the waxed plate, having mark over center of crucible and making sure that edge of crucible is in close contact with plate. Keep top surface of plate cool by means of suitable device and heat crucible for an hour at as high a temp. as practicable without melting wax (electric stove gives most satisfactory form of heat). If fluorides are present, a distinct etching will be apparent on glass where it was exposed.

27

*II. Applicable in the Presence of Silicates*

Test I may be varied by mixing small quantity of precipitated SiO<sub>2</sub> with the precipitated BaF<sub>2</sub> and applying method for detection of fluosilicates, 29 or 30.

This method is of value in the case of foods, the ash of which contains considerable quantity of SiO<sub>2</sub>. Under these circumstances H<sub>2</sub>SO<sub>4</sub> liberates SiF<sub>4</sub>, which would escape detection under 26.

**INSOLUBLE FLUORIDES**

(Fluoborates, fluosilicates, etc.)

28

**PREPARATION OF SAMPLE—OFFICIAL**

Make ca 200 g of sample alkaline with lime H<sub>2</sub>O, evaporate to dryness, and incinerate. Extract crude ash with H<sub>2</sub>O, to which has been added sufficient acetic acid to decompose carbonates; filter, ignite insoluble portion, extract with acetic acid (1 + 2), and again filter. The insoluble portion now contains CaSiO<sub>3</sub> and CaF<sub>2</sub>, while filtrate contains all the H<sub>3</sub>BO<sub>3</sub> present.

29

QUALITATIVE TEST I.<sup>11</sup>—OFFICIAL

Incinerate filter containing insoluble portion from 28, mix with a little precipitated  $\text{SiO}_2$ , transfer to short test tube attached to small U-tube containing a few drops of  $\text{H}_2\text{O}$ , and add 1–2 ml of  $\text{H}_2\text{SO}_4$ . Keep test tube in beaker of  $\text{H}_2\text{O}$  on steam bath for 30–40 min. If any F is present, the  $\text{SiF}_4$  generated will be decomposed by the  $\text{H}_2\text{O}$  in U-tube and will form a gelatinous deposit on walls of tube. Test filtrate for  $\text{H}_3\text{BO}_3$  as directed under 16. If both HF and  $\text{H}_3\text{BO}_3$  are present, it is probable that they are combined as  $\text{BF}_3$ . If, however,  $\text{SiF}_4$  is detected and  $\text{H}_3\text{BO}_3$  is not, repeat test without introducing the  $\text{SiO}_2$ , in which case formation of the silica skeleton is conclusive evidence of presence of fluosilicate. In an ash containing an appreciable quantity of  $\text{SiO}_2$ ,  $\text{H}_2\text{SO}_4$  will liberate  $\text{SiF}_4$  rather than HF. Therefore the presence of a fluosilicate, not a fluoride, is indicated.

30

## QUALITATIVE TEST II.—OFFICIAL

Incinerate filter containing insoluble portion from 28 in Pt crucible, mix with a little precipitated  $\text{SiO}_2$ , and add 1 ml of  $\text{H}_2\text{SO}_4$ . Cover crucible with watch-glass from underside of which a drop of  $\text{H}_2\text{O}$  is suspended, and heat for an hour at 70–80°, keeping watch-glass well cooled. The  $\text{H}_2\text{O}$  decomposes the  $\text{SiF}_4$  which is formed, leaving gelatinous deposit of  $\text{SiO}_2$  and etching a ring at periphery of drop of  $\text{H}_2\text{O}$ . Test filtrate for  $\text{H}_3\text{BO}_3$  as directed under 16.

## SULFUROUS ACID

31

QUALITATIVE TEST<sup>12</sup>—OFFICIAL

Add a small quantity of S-free Zn and several ml of HCl to ca 25 g of the sample (with addition of  $\text{H}_2\text{O}$ , if necessary) in 200 ml Erlenmeyer flask. The  $\text{H}_2\text{S}$  generated in presence of sulfites may be detected with Pb acetate paper. The traces of metallic sulfides occasionally present in vegetables will give the same reaction as sulfites under the conditions of the above test. Verify positive results obtained by this method by the Monier-Williams method, 32.

It is always advisable to make the quantitative determination of sulfites, owing to the danger to the test caused by traces of sulfides. A trace should not be considered sufficient indication of the presence of  $\text{SO}_2$  either as a bleaching agent or as a preservative.

## TOTAL SULFUROUS ACID

32

*Monier-Williams Method<sup>13</sup>—Official*

(Applicable in presence of other volatile S compounds.)

Connect 750 ml round-bottomed Pyrex flask (B) (Fig. 46) to sloping reflux condenser (D), the lower end of which is cut off at an angle. (Monier-Williams prefers using upright round-bottomed flask with 2 necks.) Pass  $\text{CO}_2$  from a generator thru a  $\text{Na}_2\text{CO}_3$  soln in A to remove Cl. Also connect a dropping funnel (K) to B by three-holed stopper C. Use tube E to connect upper end of condenser to 200 ml Erlenmeyer flask (F), which is followed by a Peligot tube (G). This delivery tube (E) extends to bottom of receiver. One Peligot tube has been found to be sufficient to catch traces of sulfurous acid swept thru flask F. Use rubber stoppers thruout. The receiver F contains 15 ml of pure neutral 3%  $\text{H}_2\text{O}_2$ , while the Peligot tube contains 5 ml.  $\text{H}_2\text{O}_2$  usually contains free  $\text{H}_2\text{SO}_4$ . Start with 30%  $\text{H}_2\text{O}_2$ , dilute somewhat, and neutralize with  $\text{Ba}(\text{OH})_2$  soln, using bromophenol blue soln as indicator. After the reagent has settled in the cold, filter from the  $\text{BaSO}_4$ , determine its strength by per-

manganate titration, and finally adjust to 3% strength. The bromophenol blue indicator in the  $\text{H}_2\text{O}_2$  remains unaffected for some time.

After connecting the apparatus, introduce into the flask 300 ml of  $\text{H}_2\text{O}$  and 20 ml of  $\text{HCl}$  and boil for a short time in current of  $\text{CO}_2$ . Add the food to be tested, adapting the procedure to the sort of food. Add liquids directly by means of the dropping funnel. In the case of easily transferable solids, first cool contents of flask somewhat, taking care to regulate flow of  $\text{CO}_2$  to avoid having the  $\text{H}_2\text{O}_2$  drawn up in delivery tube E. Then quickly introduce the food by removing stopper C. With semi-solid foods, requiring more time to introduce into the flask, cool flask contents by gradual immersion in cold  $\text{H}_2\text{O}$ , and wash the food in quickly with recently boiled  $\text{H}_2\text{O}$ . After introducing the food, boil mixture for 1 hour ( $1\frac{1}{2}$  hours in the case of dried

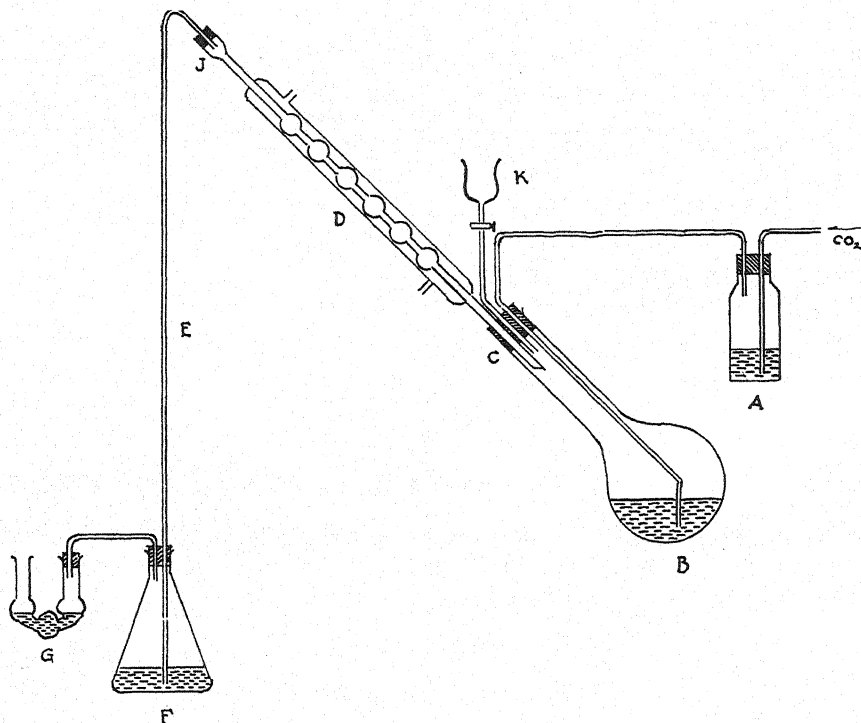


FIG. 46.—MONIER-WILLIAMS APPARATUS FOR DETERMINATION OF SULFUROUS ACID

fruits) in slow current of  $\text{CO}_2$ , stopping flow of  $\text{H}_2\text{O}$  in condenser just before the end of distillation. This causes condenser to become hot and drives over residual traces of  $\text{SO}_2$  retained in condenser. When the delivery tube just above the receiver E becomes hot to the touch, remove stopper J immediately.

Wash delivery tube and the Peligot tube contents into flask F, and titrate liquid at room temp. with 0.1 N  $\text{NaOH}$ , using bromophenol blue as indicator. The  $\text{NaOH}$  must be standardized with this indicator. Bromophenol blue is unaffected by  $\text{CO}_2$  and also gives a distinct color change in cold  $\text{H}_2\text{O}_2$ . 1 ml of 0.1 N  $\text{NaOH}$  = 3.2 mg of  $\text{SO}_2$ , so that titration of small quantities of  $\text{SO}_2$  requiring less than 0.5 ml of  $\text{NaOH}$  is not accurate. A gravimetric determination may be made after titration, the precipitation of  $\text{BaSO}_4$  being carried out at room temp. After allowing the super-

nant liquid to settle, filter, and wash residual  $\text{BaSO}_4$  3 times by decantation with boiling  $\text{H}_2\text{O}$ . Determine blank on the reagents, both by titration and gravimetrically, and correct results accordingly.

33

## FREE SULFUROUS ACID—OFFICIAL

Treat 50 ml of sample in 200 ml flask with ca 5 ml of  $\text{H}_2\text{SO}_4$  (1+3), add ca 0.5 g of  $\text{Na}_2\text{CO}_3$  to expel air, and titrate sulfurous acid with 0.02 *N* I soln, using a few ml of starch indicator, VI, 3(e). Introduce the I soln as rapidly as possible and continue addition until the blue color persists for several minutes. 1 ml of 0.02 *N* I = 0.64 mg of  $\text{SO}_2$ .

## BETA-NAPHTHOL

34

## QUALITATIVE TEST—TENTATIVE

In a separatory funnel extract 200 ml of the sample or of its aqueous extract, prepared as directed under 1(c), with 10 ml of  $\text{CHCl}_3$ . To the  $\text{CHCl}_3$  extract in test tube add a few drops of 0.5 *N* KOH soln and place in boiling water bath for 2 min. Presence of beta-naphthol is indicated by formation of deep blue color, which changes to green and then to yellow.

## ABRASTOL (ASAPROL)

35

I. SINIBALDI METHOD<sup>14</sup>—TENTATIVE

Make 50 ml of the sample alkaline with a few drops of  $\text{NH}_4\text{OH}$  and extract with 10 ml of amyl alcohol, adding ethyl alcohol if an emulsion forms. Decant the amyl alcohol, filter if turbid, and evaporate to dryness. Add to residue 2 ml of  $\text{HNO}_3$  (1+1), heat on  $\text{H}_2\text{O}$  bath until half of liquid is evaporated, and transfer to test tube with addition of 1 ml of  $\text{H}_2\text{O}$ . Add ca 0.2 g of crystallized  $\text{FeSO}_4$  and an excess of  $\text{NH}_4\text{OH}$ , dropwise, with constant shaking. If resultant precipitate is of reddish color, dissolve it in a few drops of  $\text{H}_2\text{SO}_4$ , and add crystallized  $\text{FeSO}_4$  and  $\text{NH}_4\text{OH}$  as before. As soon as a dark colored or greenish precipitate is obtained, introduce 5 ml of alcohol, dissolve precipitate in  $\text{H}_2\text{SO}_4$ , shake well, and filter. In the absence of abrastol a colorless or light yellow liquid is produced, while a red color is produced in presence of 0.01 g of abrastol.

36

II. SANGLÉ-FERRIÈRE METHOD<sup>16</sup>—TENTATIVE

Boil 200 ml of sample with 8 ml of  $\text{HCl}$  for an hour in flask fitted with reflux condenser. Abrastol is thus converted into beta-naphthol and is detected as directed under 34.

## SUCROL OR DULCIN

37

I. MORPURGO METHOD<sup>18</sup>—TENTATIVE

Evaporate about 100 ml of the sample, or of the aqueous extract prepared as directed under 1(c) and neutralized with acetic acid, to a sirupy consistency after the addition of about 5 g of basic  $\text{PbCO}_3$ , and extract the residue several times with 90% alcohol. Evaporate the alcoholic extract to dryness, extract the residue with ether, and allow the ether to evaporate spontaneously in a porcelain dish. Add 2 or 3 drops each of phenol and  $\text{H}_2\text{SO}_4$  and heat for ca 5 min. on a water bath. Cool, transfer to a test tube, and overlay with  $\text{NH}_4\text{OH}$  or  $\text{NaOH}$  soln with the least possible mixing. The presence of dulcin is indicated by the formation of a blue color at the zone of contact.

38

II. JORISSEN METHOD<sup>17</sup>—TENTATIVE

Suspend the residue from the ether extract obtained as directed under 37 in ca

5 ml of  $\text{H}_2\text{O}$ , add 2–4 ml of an approximately 10% soln of  $\text{Hg}(\text{NO}_3)_2$ , and heat for 5–10 min. on a steam bath. In the presence of sucrol a violet blue color is formed. On the addition of  $\text{PbO}_2$  the color changes to a deep violet.

### FORMIC ACID<sup>18</sup>—OFFICIAL

#### QUANTITATIVE METHOD

39

#### REAGENTS

(a) *Sodium acetate*.—Dissolve 50 g of dry Na acetate in sufficient  $\text{H}_2\text{O}$  to make 100 ml and filter.

(b) *Mercuric chloride*.—Dissolve 100 g of  $\text{HgCl}_2$  and 150 g of NaCl in sufficient  $\text{H}_2\text{O}$  to make 1 liter and filter.

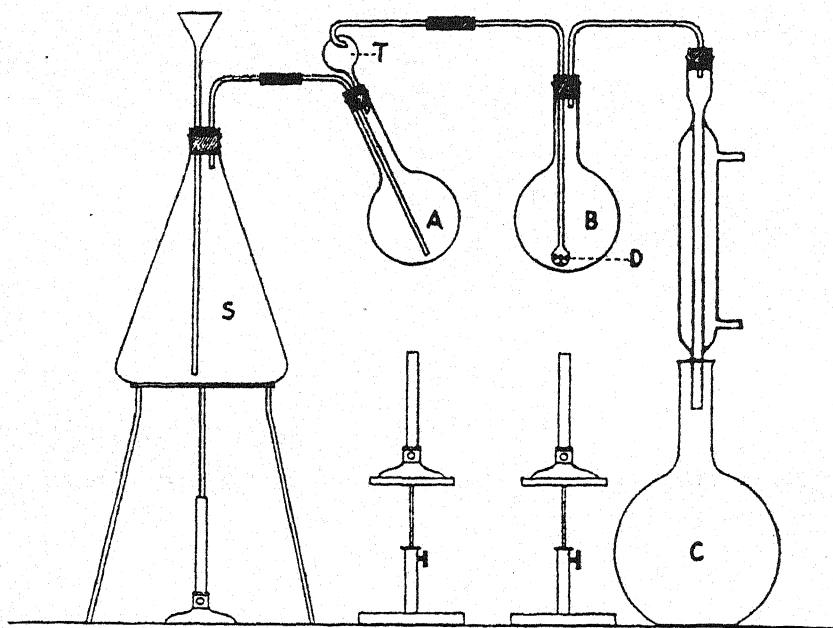


FIG. 47.—APPARATUS FOR DETERMINATION OF FORMIC ACID

40

#### APPARATUS

The apparatus required (Fig. 47) consists of steam generator (S), 300 ml flask (A) in which sample is placed, 500 ml flask (B) containing a suspension of  $\text{BaCO}_3$ , spray trap (T), condenser, and 1 liter volumetric flask (C). The tip of tube D, leading into B, consists of a bulb containing a number of small holes to break the vapor into small bubbles.

41

#### DETERMINATION

Use 50 ml of thin liquids like fruit juices; for heavy liquids and semi-solids like sirups and jams, use 50 g diluted with 50 ml of  $\text{H}_2\text{O}$ . Place sample in flask A, add 1g of tartaric acid, and connect as shown in Fig. 47, the flask B having been charged previously with a suspension of 2 g of  $\text{BaCO}_3$  in 100 ml of  $\text{H}_2\text{O}$ . If much acetic acid

is present, use sufficient  $\text{BaCO}_3$  so that at least 1 g remains at end of operation. Heat contents of flasks A and B to boiling and distil with steam from generator S, the vapor passing first thru sample in flask A, then thru the boiling suspension of  $\text{BaCO}_3$  in B, after which it is condensed and collected in volumetric flask C. Continue the distillation until 1 liter of distillate is collected, maintaining volume of liquids in flasks A and B as nearly constant as possible by heating with small Bunsen flames and avoiding charring of sample in flask A. After collecting 1 liter of distillate, disconnect apparatus and filter contents of flask B while hot, washing the  $\text{BaCO}_3$  with a little hot  $\text{H}_2\text{O}$ . Filtrate and washings should now measure ca 150 ml; if they do not, they should be boiled down to that volume. Add 10 ml of the Na acetate soln, 2 ml of 10% soln of  $\text{HCl}$ , and 25 ml of the  $\text{HgCl}_2$ . Mix thoroly and immerse container in boiling water or steam bath for 2 hours. Filter thru weighed Gooch crucible and wash precipitate thoroly with cold  $\text{H}_2\text{O}$  and finally with a little alcohol. Dry in boiling water oven for 30 min., cool, weigh, and calculate weight of  $\text{HCOOH}$  present by multiplying weight of precipitate by 0.0975. If the weight of  $\text{HgCl}$  obtained exceeds 1.5 g, repeat the determination, using more  $\text{HgCl}_2$  or a smaller quantity of sample. Conduct blank test with each new lot of reagents employed for reduction, using 150 ml of  $\text{H}_2\text{O}$ , 1 ml of 10%  $\text{BaCl}_2$  soln, 2 ml of the  $\text{HCl}$  soln, 10 ml of the Na acetate, and 25 ml of the  $\text{HgCl}_2$ , and heating mixture in boiling water or steam bath for 2 hours. Deduct weight of  $\text{HgCl}$  obtained in this blank test from that obtained in the regular determination.

## SELECTED REFERENCES

- <sup>1</sup> J. Ind. Eng. Chem., **2**, 24 (1910); **3**, 492 (1911); J. Assoc. Official Agr. Chem., **14**, 76 (1931); **16**, 77 (1933).
- <sup>2</sup> Z. Nahr. Genusssm., **19**, 137 (1910); C. A., **4**, 1523 (1910).
- <sup>3</sup> Z. Nahr. Genusssm., **18**, 577 (1909); J. Assoc. Official Agr. Chem., **17**, 193 (1934); **18**, 56 (1935); **21**, 186 (1938).
- <sup>4</sup> U. S. Dept. Agr. Div. Chem. Bull. **51**, p. 113.
- <sup>5</sup> J. Soc. Chem. Ind., **12**, 432 (1893); Chem. News, **80**, 65 (1899); Cf. Sutton, Systematic Handbook of Volumetric Analysis, 11th ed., 1924, p. 98.
- <sup>6</sup> Z. Nahr. Genusssm., **5**, 353 (1902); J. Chem. Soc., **82**, Part 2, 480 (1902).
- <sup>7</sup> Analyst, **20**, 155 (1895).
- <sup>8</sup> Ann. Farm. Chim., **27-28**, 97 (1898); Chem. centr., [1] 1152 (1898); [1] 1076 (1902); J. Soc. Chem. Ind., **17**, 697 (1898); Chem. Ztg., **26**, 246 (1902); J. Chem. Soc., **82**, Part 2, 367 (1902).
- <sup>9</sup> Bull. Assoc. chim. Belg., **11**, 211 (1897-98); Analyst, **22**, 282 (1897).
- <sup>10</sup> Chem. News, **91**, 39 (1905); Ann. Rpt. Mass. State Bd. Health, 1905, p. 498.
- <sup>11</sup> Mon. sci., (4th Ser.) **9**, Part 1, 324 (1895).
- <sup>12</sup> U. S. Dept. Agr., Div. Chem. Bull., **13**, (8), p. 1032.
- <sup>13</sup> Monier-Williams, Rpts. on Public Health and Med., Subj. No. 43. (London Min. of Health, 1927); J. Assoc. Official Agr. Chem., **12**, 120 (1929); **16**, 77 (1933); **17**, 70 (1934); **18**, 82 (1935).
- <sup>14</sup> Mon. sci., (4th Ser.) **7**, Part 2, 842 (1893).
- <sup>15</sup> Compt. rend., **117**, 796 (1893).
- <sup>16</sup> Z. anal. Chem., **35**, 104 (1896).
- <sup>17</sup> Ibid., 628.
- <sup>18</sup> Biochem. Z., **51**, 253 (1913).

## XXXIII. SPICES AND OTHER CONDIMENTS

### SPICES

1

#### PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass thru sieve having circular openings 1 mm in diameter and mix thoroly. Owing to lack of uniformity of most spices and peculiar tendency to stratify, use extreme care in weighing out portion for analysis. Stir material thoroly and weigh out 2 g sample, using spoon with capacity of ca 2 g. Dip spoonful from center of material, being careful to take approximately required quantity so as to avoid adding to or taking from portion on scale pan. In determination of starch in spices by diastase method, further reduce subsample as nearly as possible to an impalpable powder.

2

#### MOISTURE—TENTATIVE

Dry 2 g to minimum weight at 110°. From resulting loss in weight subtract quantity of volatile ether extract as determined under 9.

3

#### ASH—OFFICIAL.—*See* XXXIV, 9 or 10.

4

#### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using ash obtained under 3.

5

#### ASH INSOLUBLE IN ACID—OFFICIAL

Boil water-insoluble residue, 4, or total ash, 3, with 25 ml of HCl (1+2.5) for 5 min., collect insoluble matter on Gooch crucible or on ashless filter, wash with hot H<sub>2</sub>O, ignite until carbon-free, cool, and weigh.

6

#### CALCIUM OXIDE IN ASH—OFFICIAL

Ignite 2–4 g of sample as directed under 3, digest with hot HCl (1+2.5), evaporate to dryness, moisten dry residue with the dilute HCl, and again evaporate to dryness to render the SiO<sub>2</sub> insoluble. Treat residue with 5–10 ml of HCl, add ca 50 ml of H<sub>2</sub>O, allow to stand on water bath for a few minutes, filter, and wash insoluble residue with hot H<sub>2</sub>O. Determine CaO in combined filtrate and washings as directed under XII, 10.

7

#### NITROGEN—OFFICIAL

Proceed as directed under II, 21, 22 or 23, except in case of black and white peppers, for which use Kjeldahl-Gunning-Arnold method<sup>1</sup> (II, 23) and 1 g of sample.

8

#### NITROGEN IN NON-VOLATILE ETHER EXTRACT—OFFICIAL

(For black and white peppers.)

Extract 10 g\* of the pepper for 20 hours in continuous extraction apparatus with absolute ether, collecting extract in weighed 250 ml flask. Evaporate ether and dry first at 100° and finally to constant weight at 110°. Determine the N in weighed extract as directed under II, 23, digesting in same flask used for extraction. Crude piperine =  $N \times 20.36$ .

9

#### VOLATILE AND NON-VOLATILE ETHER EXTRACT<sup>2</sup>—OFFICIAL

Extract 2 g of ground material for 20 hours in continuous extraction apparatus with anhydrous ether. Transfer ethereal soln to weighed capsule and allow to

evaporate at room temp. Let stand 18 hours over  $\text{H}_2\text{SO}_4$  and weigh total ether extract. Heat extract gradually and then to minimum weight at  $110^\circ$ . The loss is volatile ether extract; the residue is non-volatile ether extract.

10

ALCOHOL EXTRACT<sup>3</sup>—OFFICIAL

Place 2 g of sample in 100 ml flask and fill to mark with alcohol. Stopper, shake at 30 min. intervals during 8 hours, and allow to stand 16 hours longer without shaking. Filter extract thru dry filter, evaporate 50 ml aliquot of filtrate to dryness in flat-bottomed dish on steam bath, and heat to minimum weight at  $110^\circ$ .

11

## COLD-WATER EXTRACT—TENTATIVE

(For ginger.)

Place 4 g of sample in 200 ml volumetric flask, add  $\text{H}_2\text{O}$  to mark, shake at 30 min. intervals during 8 hours, and allow to stand 16 hours longer without shaking. Filter, and evaporate 50 ml aliquot of filtrate to dryness in flat-bottomed metal dish. Dry to minimum weight at  $100^\circ$ .

12

## COPPER-REDUCING SUBSTANCES BY DIRECT INVERSION—OFFICIAL

Extract 4 g of sample with 5 successive portions of 10 ml of ether on filter that will retain completely smallest starch granules. After ether has evaporated, wash with 150 ml of alcohol, 10% by volume.

(Owing to formation of glutinous mass, which clogs filter, it is not possible to wash samples of Batavia cassia with  $\text{H}_2\text{O}$  or dilute alcohol. Therefore it is best to omit all preliminary washing in determinations made on all varieties of cassia, as well as on cassia buds and cinnamon.)

Carefully wash residue from paper into 500 ml flask with 200 ml of  $\text{H}_2\text{O}$ , using small wash bottle and gently rubbing paper with tip of finger. Hydrolyze and determine Cu reducing material, XXVII, 30. Express result in terms of starch.

13

## STARCH—OFFICIAL

Extract 4 g of finely pulverized sample with ether and 500 ml of 10% alcohol as directed under 12, and determine starch by diastase method, XXVII, 32.

14

## CRUDE FIBER—OFFICIAL

Proceed as directed under XXVII, 27, and previous to weighing remove all ether extractives by successive washings of the dry fiber with ether.

15

## TANNIN—OFFICIAL

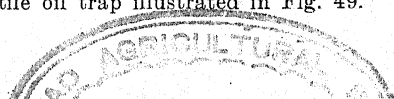
(For cloves and allspice.)

Extract 2 g of sample for 20 hours with anhydrous ether. Boil residue for 2 hours with 300 ml of  $\text{H}_2\text{O}$ , cool, make up to 500 ml, and filter. Measure 25 ml of this infusion into 2 liter porcelain dish, add 20 ml of indigo soln, XV, 31(c), and 750 ml of  $\text{H}_2\text{O}$ , and proceed as directed under XV, 32. 1 ml of 0.1 N oxalic acid = 0.00623 g of quercitannic acid, or 0.0008 g of O absorbed.

16

VOLATILE OIL<sup>4</sup>—TENTATIVE

Transfer weighed quantity of whole or ground material to 500–2000 ml round bottomed, short-necked flask in amount sufficient to yield, if possible, 2 ml or more of volatile oil. Add 3–6 times as much  $\text{H}_2\text{O}$  as material and mix uniformly. Set up apparatus, Fig. 48, using appropriate volatile oil trap illustrated in Fig. 49.





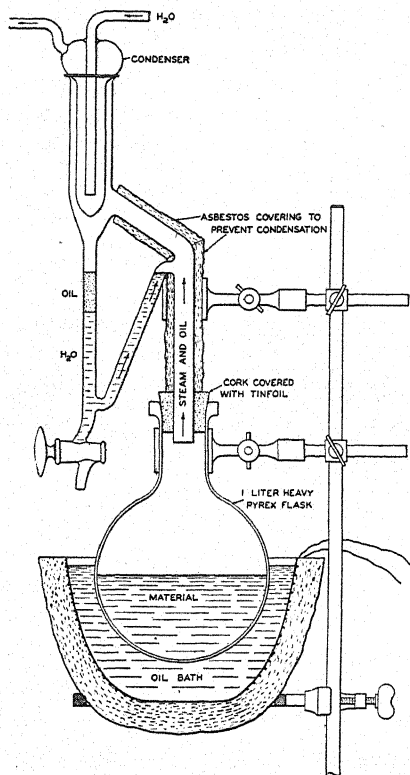


FIG. 48.—APPARATUS FOR DETERMINATION OF VOLATILE OIL

With oil bath (hydrogenated cottonseed oil is satisfactory) as source of heat, boil contents of flask slowly 4–8 hours, or until all volatile oil has been distilled, taking care to avoid escape of vapors around condenser. With spices (for example nutmeg) containing volatile oils lighter than  $H_2O$  and also fixed oils heavier than  $H_2O$ , discontinue distillation when fraction of oil obtained during a 1 hour period is heavier than  $H_2O$ .

In case of unsatisfactory separation of the volatile oil, draw off contents of trap into small separatory funnel. After separation return  $H_2O$  to trap and transfer volatile oil to graduated cylinder. Repeat procedure if necessary.

With volatile oils heavier than  $H_2O$ , after transferring oil to graduated cylinder run the  $H_2O$  with any remaining oil into small separatory funnel. Wash oil trap with 10 ml of ether and transfer washings to funnel. Shake, and withdraw the ether. Evaporate ether and drain residue into cylinder. Read quantity of volatile oil directly in cylinder and report oil in terms of ml per 100 g of spice.

Allow oil to stand until perfectly clear, or dry with minimum quantity of anhydrous  $Na_2SO_4$  and allow to settle before determining physical and chemical characteristics, 17-23.

17

#### SPECIFIC GRAVITY OF VOLATILE OIL—TENTATIVE

Determine sp. gr. at 25/25° as directed under XXXI, 3 and 4, using a 1 ml. Sprengel tube.

18

#### OPTICAL ROTATION OF VOLATILE OIL—TENTATIVE

Polarize in micropolarizing tube 50 mm long and of ca 2 mm bore with white light at 25°. (Tube may be readily filled by aid of glass tube drawn out to smaller diameter.) Report in angular degrees on basis of 100 mm tube.

19

#### REFRACTIVE INDEX OF VOLATILE OIL—OFFICIAL.—See XXXI, 8 and 9.

20

#### ACID NUMBER OF VOLATILE OIL—TENTATIVE

Add 30 ml of neutral alcohol to ca 2 g of volatile oil, accurately weighed, in 200 ml Erlenmeyer flask. Titrate with 0.1 N KOH, using 1–2 drops of 1% phenolphthalein as indicator.

$$\text{Acid No.} = \frac{\text{ml } 0.1 \text{ N KOH} \times 5.61}{\text{Wt. of volatile oil}}$$

21

## ESTER NUMBER OF VOLATILE OIL—TENTATIVE

To contents of flask after determination 20, add exactly 20 ml of 0.5 *N* KOH. Heat flask on water bath ca 2 hours, using air condenser 70–80 cm long and 5–8 mm in diameter. Determine ml of 0.5 *N* KOH used in saponification (*a*) by titrating excess with 0.5 *N* H<sub>2</sub>SO<sub>4</sub>, using 1–2 drops of phenolphthalein as indicator.

$$\text{Ester No.} = \frac{(a) \times 28.06}{\text{Wt. of volatile oil}}$$

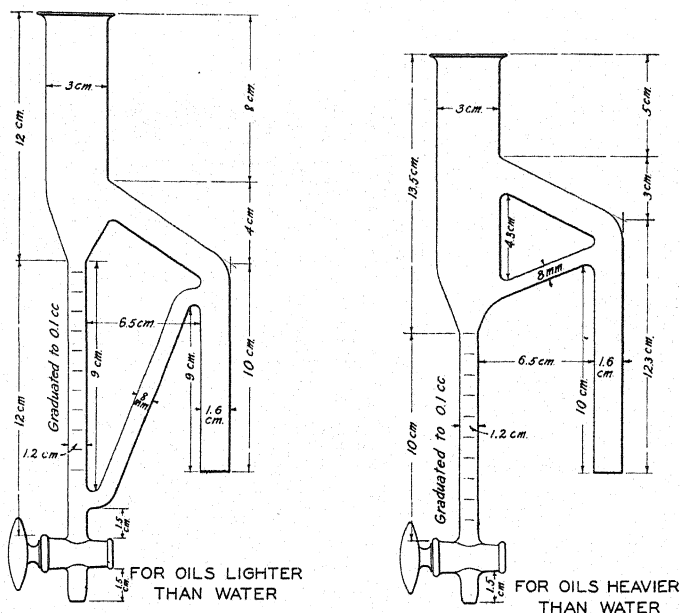


FIG. 49.—TYPES OF OIL SEPARATORY TRAPS

22

## EUGENOL IN VOLATILE OIL—TENTATIVE

Measure 2 ml of volatile oil (transfer pipet) into Babcock milk bottle, XXII, 21(a). Add 20 ml of 3% soln of KOH, shake mixture 5 min., heat for 10 min. in boiling water bath, remove, and cool to room temp. When liquids have separated completely, add sufficient KOH soln to bring residual oil within graduated portion of neck and note volume. Calculate percentage by volume from difference of volume of sample used and residual oil.

23

## KETONE AND ALDEHYDE IN VOLATILE OILS—TENTATIVE

Measure 2 ml of volatile oil (transfer pipet) into Babcock milk bottle, XXII, 21(a). Add 10 ml of saturated soln of Na<sub>2</sub>SO<sub>3</sub> and a few drops of 1% phenolphthalein soln. Heat in bath containing boiling H<sub>2</sub>O, and shake flask repeatedly, neutralizing mixture occasionally with a few drops of saturated soln of NaHSO<sub>3</sub>. If no coloration appears upon adding a few drops of phenolphthalein soln and heating for 30 min., cool to room temp. When liquids have separated completely, add sufficient Na<sub>2</sub>SO<sub>3</sub> soln to bring residual oil within graduated portion of neck and note volume.

Calculate percentage by volume from difference of volume of sample used and residual oil.

24

VOLATILE OIL AND RESIN IN GINGER<sup>a</sup>—TENTATIVE

Place 50 g of ground ginger in Soxhlet extractor and extract completely, using ether as solvent (ca 4 hours). Transfer extract to 300 ml flask and evaporate off ether on steam bath until solvent is no longer detected. Add 50 ml of  $H_2O$  to residue and determine yield of volatile oil (using trap for oils lighter than  $H_2O$ ) and determine sp. gr., optical rotation, refractive index, acid and ester numbers as directed under 17–21.

Transfer residue in flask to separatory funnel and extract resin with ether. Transfer to tared beaker, evaporate ether on steam bath, and dry to constant weight in vacuum desiccator.

25

VOLATILE OIL IN MUSTARD SEED<sup>a</sup>—OFFICIAL

Place 5 g of ground seed (No. 20 powder) in 200 ml flask, add 100 ml of  $H_2O$ , stopper tightly, and macerate for 2 hours at ca  $37^\circ$ . Add 20 ml of alcohol and distil ca 60 ml into 100 ml volumetric flask containing 10 ml of  $NH_4OH$  (1+2), taking care that end of condenser dips below surface of soln. Add 20 ml of 0.1  $N$   $AgNO_3$  soln to distillate, set aside overnight, heat to boiling on water bath in order to agglomerate  $(Ag)_2S$ , cool, make up to 100 ml with  $H_2O$ , and filter. Acidify 50 ml of filtrate with ca 5 ml of  $HNO_3$  and titrate with 0.1  $N$   $NH_4CNS$ , using 5 ml of 10%  $FeNH_4(SO_4)_2 \cdot 12H_2O$  as indicator. 1 ml of 0.1  $N$   $AgNO_3$  consumed = 0.004956 g of allyl isothiocyanate.

26

## VOLATILE OIL IN MARJORAM AND SAGE—OFFICIAL.—See 16.

IODINE NUMBER OF PAPRIKA OIL<sup>a</sup>—OFFICIAL, FIRST ACTION

27

(Qualitative test for presence of foreign oil.)

Transfer 10 g of well-mixed ground sample to 200 ml glass-stoppered flask and add 100 ml of  $CHCl_3$  from pipet, rotating while adding first 50 ml. Let stand 1 hour, shake, and filter thru  $12\frac{1}{2}$  cm fluted filter paper. Pipet off successively two 10 ml portions, using same pipet. Transfer one portion to weighed crystallizing dish,  $50 \times 35$  mm, and evaporate solvent by placing dish on steam bath. Dry dish and contents at  $100^\circ$  for 1 hour, cool in air, and weigh. Use weight obtained in calculating I number. Transfer other portion to suitable glass-stoppered flask or bottle for determination of I number, XXXI, 19, allowing 30 min. for halogen absorption. Calculate I number of  $CHCl_3$  extract. The I number of pure paprika thus obtained should be not less than 130.

## MICROSCOPIC EXAMINATION—TENTATIVE

28

## GENERAL

Adulterants of vegetable origin in spices are best detected by means of the microscope. A general knowledge of vegetable histology and the microscopic appearance of the spices and spice adulterants is essential. Some of the standard works<sup>9</sup> on these subjects are listed under the selected references.

29

## REAGENTS

(a) *Ammoniacal copper soln (Schweitzer's reagent)*. Add slowly a soln of  $CuSO_4$  to a soln of  $NaOH$ , leaving slight excess of  $NaOH$ ; separate by filtration the precipitate of  $Cu(OH)_2$  that forms and wash it thoroly with  $H_2O$ . Dissolve wet precipi-

tate in  $\text{NH}_4\text{OH}$  with aid of heat, cool, and filter. Prepare immediately before use and keep in dark.

(b) *Potassium hydroxide soln.*—Dissolve 5 g of KOH in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(c) *Chloral hydrate.*—Dissolve 8 parts by weight of crystals in 5 parts of  $\text{H}_2\text{O}$ .

(d) *Acidified chloral hydrate-glycerol soln.*—Dissolve 45 g of crystals of chloral hydrate in 25 ml of HCl (1+8) and 10 ml of glycerol.

(e) *Schultze's mixture.*—Mix crystallized  $\text{KClO}_3$  with  $\text{HNO}_3$  as needed.

(f) *Iodine-potassium iodide soln (iodine soln).*—Dissolve 0.05 g of I and 0.2 g of KI in 15 ml of  $\text{H}_2\text{O}$ .

(g) *Chlor-zinc iodine soln.*—Dissolve 100 g of  $\text{ZnCl}_2$  in 60 ml of  $\text{H}_2\text{O}$  in glass-stoppered bottle and add 20 g of KI and 0.5 g of I crystals. Leave a few crystals of I in bottle to insure saturation, and allow soln to stand a few hours before using. (Soln will keep for months. If color developed in tissue is too deep a blue, very slight dilution of reagent is advisable.)

(h) *Ferric acetate or chloride soln.*—Use freshly prepared 1% aqueous soln.

(i) *Alkanet tincture.*—Macerate 20 g of alkanet root for several days with 100 ml. of alcohol.

(j) *Safranine soln.*—Prepare saturated aqueous soln and dilute as needed.

(k) *Mayer's reagent (mercuric-potassium iodide soln).*—Dissolve 1.36 g of  $\text{HgCl}_2$  in 60 ml of  $\text{H}_2\text{O}$  and 5 g of KI in 10 ml of  $\text{H}_2\text{O}$ ; mix these two solns and dilute to 100 ml.

(l) *Millon's reagent.*—See XXVII, 12.

## 30

## APPARATUS

(a) *Dissecting microscope or hand lens.*

(b) *Compound microscope.*—Provided with objectives and oculars capable of giving range of ca 4 different magnifications varying from 75 to 500 diameters, a double or triple nosepiece, and a substage condenser. An eyepiece micrometer, a polarizing apparatus, and a mechanical stage are desirable for some special types of work.

(c) *Sieves.*—A series of standard mesh sieves varying from 10 to 100 meshes per inch and a sieve having circular openings 1 mm in diameter.

(d) *Slides, cover glasses, needles, forceps, etc.*

## 31

## PREPARATION OF SAMPLE

Reduce one portion to fine powder in mortar. Separate another portion into several grades of fineness by sieves of different mesh or by jarring on sheet of paper. In coarser grades fragments of suspicious nature may often be seen with naked eye or under simple microscope; these should be picked out for subsequent examination under compound microscope.

## 32

## EXAMINATION

Mount a small quantity of ground sample in  $\text{H}_2\text{O}$  and examine under compound microscope with both ordinary and polarized light. This gives general information as to nature of material and serves for detection and identification of starch granules and various tissues. Place a small drop of the I-KI soln at edge of cover-glass, draw it into preparation by means of piece of filter paper placed at opposite edge of cover-glass, and examine again. Starch granules will be colored blue or blue-black; cellulose, yellow; and proteins, either brown or yellow.

In manner just described draw a little of the KOH soln under cover-glass and again examine. This treatment gelatinizes starch granules, dissolves proteins, saponifies fats, and in other ways clears preparation. It also imparts to tannins a

reddish color. If this treatment does not clear tissues satisfactorily, treat fresh portion for short time with the acidified chloral hydrate-glycerol soln, or for some hours with the chloral hydrate soln.

Examine also the crude fiber obtained in the chemical analysis, as in this material stone cells and other tissues are shown distinctly.

To isolate stone cells, bast fibers, and other thick-walled cells, macerate portion of sample in Schultze's mixture, using such proportions of  $\text{KClO}_3$  and  $\text{HNO}_3$  and heating for such time as will secure desired results.

To distinguish cellulose from infiltrated substances (lignin, suberin, etc.), add freshly prepared chlor-zinc iodine soln to water mount. Cellulose is colored blue, and infiltrated substances are yellow.

To distinguish fats, oils, essential oils, and resins from other cell contents, treat for an hour with alkanet tincture diluted with equal volume of  $\text{H}_2\text{O}$ , which imparts to these substances a deep red color, or treat with ether, which dissolves them. Treat also with alcohol, which dissolves essential oils and resins but does not perceptibly affect fats and oils.

Test for proteins by warming cautiously on slide with a drop of freshly prepared Millon's reagent. The proteins are partially decomposed, acquiring gradually a brick red color. If it is desired to study the form of the aleurone (protein) granules, which in some plants are quite as characteristic as starch granules, prepare a mount in pure glycerol or oil.

Test for tannins and tissues impregnated with them by adding 1% Fe acetate or chloride soln. Both of these reagents give a green or blue color with tannins, but the ferric acetate acts more slowly and is to be preferred.

Crystals of Ca oxalate<sup>10</sup> are recognized by their characteristic forms and by their behavior to polarized light. To distinguish Ca oxalate from  $\text{CaCO}_3$ , treat with acetic acid, which does not affect the oxalate but dissolves the carbonate with effervescence. Both are soluble in HCl.

Powdered charcoal and charred shells resist the bleaching action of potash, chloral hydrate, and Schultze's mixture.

### PREPARED MUSTARD

33

#### PREPARATION OF SAMPLE—OFFICIAL

Transfer entire contents of container to dish sufficiently large to permit thorough stirring and make whole mass homogeneous. Preserve in bottle having tightly fitting glass stopper. Stir well each time before removing portion for analysis.

34

#### SOLIDS—OFFICIAL

Weigh 5 g of sample into flat-bottomed Pt dish; distribute evenly over bottom of dish with a little  $\text{H}_2\text{O}$ , place on steam bath until mixture appears dry, and heat in oven at  $100^\circ$  to constant weight.

35

#### ASH—OFFICIAL

Ignite dry residue, 33, as directed under XXXIV, 9 or 10.

36

#### SALT—OFFICIAL

Determine Cl in ash, 35, as directed under XII, 35 or 37.

37

#### ETHER EXTRACT—TENTATIVE

Weigh 10 g of sample into Si, Al, or porcelain drying dish and mix with ca 30 g of sand. Heat on water bath until mixture appears dry and complete drying in water

oven. Grind until all lumps are broken up, and determine ether extract as directed under XXVII, 22.

38

## TOTAL NITROGEN "PROTEIN"—OFFICIAL

Determine N as directed under II, 21, 22, or 23, using 5 g of sample. "Protein" =  $N \times 6.25$ .

39

## ACIDITY—OFFICIAL

Weigh 10 g of sample into 200 ml volumetric flask, dilute to mark with  $H_2O$ , shake, filter thru dry paper, and determine acidity in 100 ml by titration with 0.1 N alkali, using phenolphthalein indicator. Express result as acetic acid. 1 ml of 0.1 N alkali = 0.0060 g of acetic acid.

40

## COPPER-REDUCING SUBSTANCES BY DIRECT INVERSION—OFFICIAL

Proceed as directed under XXVII, 30, except to treat directly 10 g of sample (without previous washing or extraction) with 200 ml of  $H_2O$  and 20 ml of HCl (sp. gr. 1.125), and to make up soln to 250 ml after neutralizing and before filtering and drawing off aliquot. In analyses of samples containing starch, do not have quantity of dextrose present in aliquot taken for reducing sugar determination exceed maximum permitted for that determination. Express result in terms of starch.

41

CRUDE FIBER<sup>11</sup>—OFFICIAL

Weigh 10 g of sample and transfer to 8 oz nursing bottle with 50 ml of alcohol, stopper, and shake vigorously. Add 40 ml of ether, shake, and let stand ca 5 min. with occasional shaking. Centrifuge and decant alcohol-ether mixture. Treat twice more with 40 ml portions of ether, shaking, centrifuging, and decanting as before. Rest bottle on its side for short time, without heat, to allow most of ether to evaporate. Transfer material to 500 ml Erlenmeyer flask, using 200 ml of boiling  $H_2SO_4$ , XXVII, 25(a), and proceed as directed under XXVII, 27, but in addition wash fiber with successive portions of ether previous to drying and weighing.

If preferred, treat sample with alcohol and ether in small beaker, transfer to hardened 11 cm filter paper, wash several times with ether, and transfer to 500 ml Erlenmeyer flask with 200 ml of boiling  $H_2SO_4$ .

42

## COLORING MATTER—TENTATIVE.—See XXI.

43

## PRESERVATIVES—OFFICIAL.—See XXXII.

MAYONNAISE AND SALAD DRESSING<sup>12</sup>

44

## PREPARATION OF SAMPLE—TENTATIVE

Before removing portion of sample for analysis and each time subsequent portion is removed, if sample has stood for any appreciable length of time, mix until it is homogeneous. For various determinations take approximately quantity directed and weigh. (A Bailey weighing buret<sup>13</sup> will be found convenient.)

45

TOTAL SOLIDS<sup>14</sup>—OFFICIAL

Use 2 g sample and proceed as directed in XXIII, 2.

46

## REDUCING SUGARS BEFORE INVERSION—TENTATIVE

Weigh 20 g of sample into wide-mouthed, 4 oz bottle and extract oil by adding ca 80 ml of petroleum benzin, shaking, and centrifuging. Draw off as much as possible of petroleum benzin soln (conveniently done by using suction and short-

stemmed pipet), and repeat treatment with petroleum benzin until all oil has been removed (indicated by absence of color in solvent—usually four extractions are required). Reserve ether soln for identification of oil. Remove petroleum benzin from residue with current of air and transfer residue with  $H_2O$  to 100 ml volumetric flask. Add 5–10 ml of fresh soln of  $HPO_3$  (5 g of the transparent (any white coating due to exposure of  $HPO_3$  removed by rinsing with  $H_2O$ ) lumps or sticks dissolved in cold  $H_2O$  and diluted to 100 ml), mix thoroly, dilute to volume, and filter. Transfer 80 ml of filtrate, or as large an aliquot as possible, to 100 ml flask; neutralize with soln of  $NaOH$  (1+1), using phenolphthalein indicator; cool, dilute to mark, and determine reducing sugars on aliquot as directed under XXXIV, 39. Calculate to invert sugar.

With dressings, particularly those containing starch, that cannot be clarified by above method, remove oil as directed under XXIII, 8 or 9, using 1 ml of  $NH_4OH$  and 5 ml of alcohol for each gram of sample; transfer residue to 250 ml flask with alcohol, 50% by volume, and proceed as directed under XXVII, 28 and XXXIV, 38 and 39.

47

## REDUCING SUGARS AFTER INVERSION—TENTATIVE

Invert aliquot of the soln, 46, as directed under XXXIV, 24(b) or (c), and determine reducing sugars in inverted soln as directed under XXXIV, 38 and 39. Calculate to invert sugar.

48

## SUCROSE—TENTATIVE

Subtract percentage of invert sugar obtained before inversion, 46, from that obtained after inversion, 47, and multiply difference by 0.95.

49

TOTAL ACIDITY<sup>14</sup>—OFFICIAL

Weigh ca 15 g of sample into 500 ml Erlenmeyer flask, dilute to ca 200 ml, and shake until all lumps of dressing are thoroly broken up. Titrate with 0.10 *N*  $NaOH$ , using neutral phenolphthalein, and calculate as acetic acid. In order to recognize the end point, have duplicate sample at hand so that, by comparison, first change of color may be noted.

50

TOTAL NITROGEN<sup>14</sup>—OFFICIAL

Weigh ca 15 g of sample into 500 ml Kjeldahl flask and place on steam bath until egg is thoroly cooked and oil separates readily. Cool, and add ca 50 ml of petroleum benzin; mix, and pour off benzin soln thru small filter. Repeat benzin treatment twice, rinsing out as much oil as possible. Wash filter with petroleum benzin and add filter paper to sample in flask. Determine *N*, using 35 ml of  $H_2SO_4$  for digestion as directed in II, 23.

51

TOTAL PHOSPHORIC ACID ( $P_2O_5$ )<sup>14</sup>—OFFICIAL

Use 10 g sample and proceed as directed in XXIII, 13 and 14, except to use Pt dish in place of beaker and to burn off oil before ashing in muffle.

52

TOTAL FAT<sup>14</sup>—TENTATIVE

Use 2 g sample and proceed as directed under XXIII, 8 and 9.

53

CALCULATION OF COMPOSITION<sup>14</sup>—TENTATIVE

When  $P$  = % total  $P_2O_5$  and  $N$  = % total nitrogen, then

% yolk =  $75.69 P - 1.802 N$ ;

% white =  $60.80 N - 114.59 P$ ;

% total egg = % yolk + % white;

$$\% \text{ white in egg component} = \frac{\% \text{ white}}{\% \text{ total egg}} \times 100;$$

Vegetable oil = total fat - (yolk  $\times$  0.3188);

Vinegar (4% acid strength) = total acidity as acetic  $\times$  25;

Minor constituents (sugar, salt, spices, stabilizers) = total solids - (yolk  $\times$  0.5047) - (white  $\times$  0.1221) - vegetable oil; and

Added water = 100% - total egg - vegetable oil - vinegar - minor constituents.

54

## IDENTIFICATION OF OIL—TENTATIVE

Proceed as directed under XXXI, using oil obtained by evaporating petroleum benzin extracts from determination of reducing sugars, 46.

55

GUMS IN MAYONNAISE AND FRENCH DRESSING<sup>15</sup>—TENTATIVE

Transfer 100 g into 250 ml beaker, add 35–40 ml of hot H<sub>2</sub>O, and mix thoroly. Heat to 65–70° in water bath, add 10 ml of 50% trichloroacetic acid soln in H<sub>2</sub>O, and maintain at 65–70° until the emulsion shows signs of breaking (in no case over 10 min.). Transfer mixture to 8 oz nursing bottle, insert pipet guard,<sup>16</sup> and centrifuge 15–20 min. at ca 1200 r.p.m. (This should separate mixture into lower aqueous layer and upper oily layer, with layer of curd between. If separation does not occur, add 30–40 ml of toluene, mix, and repeat the centrifuging.) By means of pipet inserted thru pipet guard remove as much of aqueous layer as possible and filter it into 600 ml beaker. Add 5 volumes of alcohol and allow mixture to stand overnight to precipitate the gums.

Decant or pipet off sufficient alcohol to leave not over 225 ml, transfer contents of beaker to 8 oz nursing bottle, centrifuge until gum settles to bottom, and decant supernatant alcohol as completely as possible. Dissolve residue in not over 1.5 oz of hot H<sub>2</sub>O, add 1 or 2 ml of acetic acid, and reprecipitate by adding alcohol to 8 oz mark on nursing bottle. Let stand overnight, or until precipitate becomes flocculent, centrifuge at 1200 r.p.m., and decant alcohol. A precipitate at this point indicates gums. This may be confirmed by the following procedure:

Add 35 ml of hot H<sub>2</sub>O to precipitate in nursing bottle, transfer to small beaker, add 5 ml of HCl, and boil gently 2 min. to hydrolyze gums to sugars. This soln may now be used for various qualitative tests for monosaccharide sugars, as follows:

1. Transfer 1 ml of hydrolyzed gum soln to test tube, neutralize with approximately 2 N NaOH, using litmus paper as reagent, remove litmus paper, add 5 ml of Benedict's qualitative sugar soln, XXII, 127, and boil vigorously 1–2 min. Allow to cool spontaneously. A voluminous precipitate, which may be green, yellow, or red, indicates reducing sugars.

2. Molisch test.—Transfer 5 ml of the hydrolyzed gum soln to test tube, and add 2 drops of 15% soln of alpha naphthol in alcohol. Incline tube and slowly pour down inner side 3–5 ml of H<sub>2</sub>SO<sub>4</sub> so that the two layers will not mix. A reddish-violet zone at point of contact indicates carbohydrates. (A 5% soln of thymol in alcohol may be substituted for alpha naphthol.)

If sufficient soln remains, divide into two equal parts and apply following tests:

3. Seliwanoff test.—Heat the hydrolyzed gum soln to boiling, and add a few mg of resorcinol. A red color indicates hexoses.

4. Tollens test.—Heat the hydrolyzed gum soln to boiling, and drop in a few crystals of phloroglucinol. A red or deep amber color indicates pentoses. Certain other sugars (as galactose) also give a positive reaction.



VINEGARS<sup>15</sup>

(Unless otherwise directed, express results as grams per 100 ml.)

56

## PHYSICAL EXAMINATION—OFFICIAL

Note appearance, color, odor, and taste.

57

## PREPARATION OF SAMPLE—OFFICIAL

Mix thoroly and filter before proceeding with analysis.

58

## SPECIFIC GRAVITY—OFFICIAL

Determine specific gravity at 20/20° by means of pycnometer, XIV, 3.

59

## SOLIDS—OFFICIAL

Measure 10 ml of sample into weighed, flat-bottomed Pt dish having bottom diameter of 50 mm, evaporate on boiling water bath for 30 min., and dry for exactly 2.5 hours in water oven at temp. of boiling H<sub>2</sub>O. Cool in desiccator and weigh. To obtain concordant results, it is necessary to use a dish of size and shape stated and to dry for exactly time specified.

60

## ASH—OFFICIAL

Measure 25 ml of the vinegar into weighed Pt dish, evaporate to dryness on water or steam bath, and heat in muffle at 500–550° for 30 min. Break up charred mass in Pt dish, add hot H<sub>2</sub>O, filter thru ashless filter, and wash *thoroly* with H<sub>2</sub>O. Return filter and contents to dish, dry, and heat at ca 525° for 30 min., or until all carbon is burned off. Add filtrate, evaporate to dryness, and heat at ca 525° for 15 min. Cool in desiccator and weigh (Weight A). Reheat in muffle at ca 525° for 5 min., and cool for not more than 1 hour in desiccator containing efficient desiccant. Put no more than 2 dishes, preferably only 1, in desiccator at one time. Place Weight A on balance pan before removing dish from desiccator, and weigh rapidly to a milligram. Calculate total ash from last weight.

61

## SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Treat the ash, 60, as directed under XXXIV, 13.

62

## ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using soluble ash obtained under 61. Express result as number of ml of *N* acid required to neutralize soluble ash from 100 ml of the vinegar. If relationship of ash to alkalinity of soluble ash is abnormal, study composition of the ash, especially as to content of chlorides, sulfates, phosphates, and alkalis.<sup>18</sup>

63

## SOLUBLE PHOSPHORIC ACID—OFFICIAL

Proceed as directed under II, 9 or 12, using soln obtained under 62. Express result as mg of P<sub>2</sub>O<sub>5</sub> in 100 ml of vinegar.

64

## INSOLUBLE PHOSPHORIC ACID—OFFICIAL

Dissolve water-insoluble ash, 61, in ca 50 ml of boiling HNO<sub>3</sub> (1+8) and proceed as directed under II, 9 or 12. Express result as mg of P<sub>2</sub>O<sub>5</sub> in 100 ml of vinegar.

65

## TOTAL ACIDS—OFFICIAL

Dilute 10 ml of sample with recently boiled and cooled  $\text{H}_2\text{O}$  until it appears slightly colored and titrate with 0.5 *N* alkali, using phenolphthalein indicator. 1 ml of 0.5 *N* alkali = 0.030 g of acetic acid.

66

## NON-VOLATILE ACIDS—OFFICIAL

Measure 10 ml of the vinegar into 200 ml porcelain casserole, evaporate just to dryness, add 5–10 ml of  $\text{H}_2\text{O}$ , and again evaporate; repeat until at least 5 evaporations have been made. Add ca 200 ml of recently boiled and cooled  $\text{H}_2\text{O}$  and titrate with 0.1 *N* alkali soln, using phenolphthalein indicator. 1 ml of 0.1 *N* alkali = 0.0060 g of acetic acid.

67

## VOLATILE ACIDS—OFFICIAL

Subtract quantity of non-volatile acids, 66, from quantity of total acids, 65.

68

## TOTAL REDUCING SUBSTANCES BEFORE INVERSION—OFFICIAL

Measure 25 ml of sample into 50 ml volumetric flask and add enough NaOH soln (1+1) nearly to neutralize the acid. Cool, dilute to mark with  $\text{H}_2\text{O}$ , and determine reducing substances in 20 ml of the soln as directed under XXXIV, 38. If quantity of reducing substances is very small, use 40 ml of the soln. Calculate result as invert sugar (for malt vinegar as dextrose).

69

## TOTAL REDUCING SUBSTANCES AFTER INVERSION—OFFICIAL

Invert 25 ml of sample in 50 ml volumetric flask with 5 ml of HCl, as directed under XXXIV, 24(b) or (c). Neutralize with NaOH soln (1+1) and determine reducing substances as directed under XXXIV, 38.

70

## NON-VOLATILE REDUCING SUBSTANCES (SUGAR)—OFFICIAL

(Useful in calculating the non-sugar solids.)

Evaporate 50 ml of sample on steam or water bath to sirupy consistency, add 10 ml of  $\text{H}_2\text{O}$ , and evaporate again. Repeat with 10 ml of  $\text{H}_2\text{O}$ . Transfer residue to 100 ml volumetric flask with ca 50 ml of warm  $\text{H}_2\text{O}$ . Cool; invert with 10 ml of HCl as directed under XXXIV, 24(b) or (c); nearly neutralize with NaOH soln (1+1); cool, dilute to mark with  $\text{H}_2\text{O}$ , and determine reducing substances in 20 ml or 40 ml, depending on quantity present, as directed under XXXIV, 38. Calculate result as invert sugar (for malt vinegar as dextrose). If results for total reducing substances before and after inversion show absence of sucrose, the inversion may be omitted.

71

VOLATILE REDUCING SUBSTANCES<sup>12</sup>—OFFICIAL

When sucrose is absent, subtract quantity of non-volatile reducing substances, 70, from the mean of the total reducing substances before inversion, 68, and after inversion, 69. When sucrose is present, subtract the quantity of non-volatile reducing substances, 70, from the quantity of total reducing substances after inversion, 69.

72

## ALCOHOL—OFFICIAL

Measure 100 ml of sample into round-bottomed distillation flask. Make faintly alkaline with NaOH soln (1+1), distil almost 50 ml, dilute to 50 ml at the temp. of the sample, and determine sp. gr. at 20/20° by means of pycnometer, XIV, 3. Obtain from Table 19, XLIII, percentage by volume. Undue foaming may be obviated by adding small piece of paraffin, free from volatile constituents.

GLYCEROL<sup>20</sup>—OFFICIAL

73

## REAGENTS

(a) *Strong potassium dichromate soln.*—Dissolve 74.55 g of dry, recrystallized  $K_2Cr_2O_7$  in  $H_2O$ ; add 150 ml of  $H_2SO_4$ ; cool, and dilute with  $H_2O$  to 1 liter at  $20^\circ$ . 1 ml of this soln = 0.01 g of glycerol. Owing to high coefficient of expansion of this strong soln it is necessary to make all volumetric measurements of the soln at the same temp. as that at which it was diluted to volume.

(b) *Dilute potassium dichromate soln.*—Measure 25 ml of the strong  $K_2Cr_2O_7$  soln at  $20^\circ$  into 500 ml volumetric flask and dilute to mark with  $H_2O$  at room temp. 20 ml of this soln = 1 ml of (a).

(c) *Ferrous ammonium sulfate soln.*—Dissolve 30 g of crystallized ferrous ammonium sulfate in  $H_2O$ , add 50 ml of  $H_2SO_4$ , cool, and dilute with  $H_2O$  to 1 liter at room temp. 1 ml of this soln = approximately 1 ml of (b). As its value changes slightly from day to day, it must be standardized against (b) whenever used.

(d) *Diphenylamine indicator.*—Dissolve 1 g of diphenylamine in 100 ml of  $H_2SO_4$ .

(e) *Retarder.*—Dilute 150 ml of sirupy phosphoric acid with 600 ml of  $H_2O$ , and add 250 ml of  $H_2SO_4$ .

(f) *Milk of lime.*—Introduce 150 g of  $CaO$ , selected from clean hard lumps, prepared preferably from marble, into large porcelain or iron dish; slake with  $H_2O$ , cool, and add sufficient  $H_2O$  to make 1 liter.

(g) *Silver carbonate.*—Dissolve 0.1 g of  $Ag_2SO_4$  in ca 50 ml of  $H_2O$ , add an excess of  $Na_2CO_3$  soln, allow precipitate to settle, and wash with  $H_2O$  several times by decantation until washings are practically neutral. This reagent must be freshly prepared immediately before use.

74

## DETERMINATION

Make evaporations on water bath maintained at temp. of  $85-90^\circ$ . The area of dish exposed to bath should not be greater in circumference than that covered by the liquid inside.

Evaporate 100 ml of the vinegar to 5 ml, add 20 ml of  $H_2O$ , and again evaporate to 5 ml to expel acetic acid. Treat residue with ca 5 g of 40-mesh sand and 15 ml of the milk of lime and evaporate almost to dryness, with frequent stirring, avoiding formation of dry crust or evaporation to complete dryness. Treat moist residue with 5 ml of  $H_2O$ ; rub to homogeneous paste; add slowly 45 ml of absolute alcohol, washing down sides of dish to remove adhering paste; and stir thoroly. Heat mixture on water bath, with constant stirring, to incipient boiling; transfer to suitable vessel and centrifuge. Decant clear liquid into porcelain dish and wash residue with several small portions of hot alcohol, 90% by volume, by aid of centrifuge. (If centrifuge is not available, decant liquid thru folded filter into porcelain dish. Wash residue repeatedly with small portions of hot 90% alcohol, twice by decantation, and then by transferring all material to filter. Continue washing until filtrate amounts to 150 ml.) Evaporate to sirupy consistency, add 10 ml of absolute alcohol to dissolve residue, and transfer to 50 ml glass-stoppered cylinder, washing dish with successive small portions of absolute alcohol until volume of soln is 20 ml. Add 3 portions of 10 ml each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru filter, and wash cylinder and filter with mixture of 2 volumes of absolute alcohol and 3 of anhydrous ether. If heavy precipitate has formed in cylinder, centrifuge at low speed, decant clear liquid, and wash 3 times with 20 ml portions of the alcohol-ether mixture, shaking mixture thoroly each time and separating precipitate by means of centrifuge. Wash paper with the alcohol-ether

mixture and evaporate filtrate and washings on water bath to ca 5 ml; add 20 ml of H<sub>2</sub>O, and again evaporate to 5 ml; again add 20 ml of H<sub>2</sub>O and evaporate to 5 ml; finally add 10 ml of H<sub>2</sub>O and evaporate to 5 ml.

These evaporations are necessary to remove all the ether and alcohol, and when conducted at 85–90° they result in no loss of glycerol if the concentration of the latter is less than 50%.

Transfer residue with hot H<sub>2</sub>O to 50 ml volumetric flask, cool, add the Ag<sub>2</sub>CO<sub>3</sub> prepared from 0.1 g of Ag<sub>2</sub>SO<sub>4</sub>, shake, and allow to stand 10 min. Add 0.5 ml of basic Pb acetate soln, XXXIV, 19(a); shake occasionally, and allow to stand 10 min. Make up to mark, shake well, and filter, rejecting first portion of filtrate. Pipet 25 ml of clear filtrate into 250 ml volumetric flask.

Add 1 ml of H<sub>2</sub>SO<sub>4</sub> to precipitate excess of Pb and then 30 ml of Reagent (a). Add carefully 24 ml of H<sub>2</sub>SO<sub>4</sub>, rotating flask gently to mix contents and avoid violent ebullition, and then place in *boiling* water bath for exactly 20 min. Remove flask from bath, dilute, cool, and make up to mark at room temp. The quantity of strong dichromate soln used must be sufficient to leave excess of ca 12.5 ml at end of oxidation. (Quantity given above (30 ml) is sufficient for ordinary vinegar containing ca 0.35 g or less of glycerol per 100 ml.)

Standardize the ferrous ammonium sulfate soln by pipetting 20 ml into 250 ml beaker, adding 20 ml of the retarder, 4 drops of the indicator, and ca 100 ml of H<sub>2</sub>O. Titrate with the dilute potassium dichromate soln until liquid assumes dark green color, then add the dichromate slowly dropwise, stirring continuously, until color changes from blue gray to deep violet. Designate the ml of dilute dichromate soln used as (a). In place of the dilute dichromate soln, substitute a buret containing the oxidized glycerol with an excess of the strong dichromate soln and titrate 20 ml of the ferrous ammonium sulfate soln as before, designating the ml used as (b).

From figures obtained calculate glycerol by following formula:

$$G = \left( D - \frac{250(a)}{20(b)} \right) 0.02, \text{ in which}$$

$G$  = g of glycerol per 100 ml of vinegar, and  $D$  = ml of the strong potassium dichromate soln used to oxidize the glycerol.

75

## COLOR—OFFICIAL

Determine depth of color in Lovibond tintometer by good reflected daylight, using  $\frac{1}{2}$  or 1" cell and the brewer's scale. Report result in terms of  $\frac{1}{2}$ " cell and so state.

76

COLOR REMOVED BY FULLERS' EARTH<sup>21</sup>—TENTATIVE

To 50 ml of sample add 10 g of fullers' earth, shake at intervals for 30 min., and filter thru folded filter. Place as much of filtrate as is available into colorimeter tube and place equal volume of original sample in corresponding tube; dilute both with H<sub>2</sub>O to volume of 50 ml and compare colors. Express result as percentage of color removed. (Not all fullers' earth is satisfactory for this test. The efficiency of the reagent should be determined on a sample of distilled vinegar known to be colored with caramel.)

77

POLARIZATION<sup>22</sup>—TENTATIVE

Whenever possible, polarize in 200 mm tube without decolorizing. Report results on basis of 200 mm tube in degrees Ventzke. When necessary, decolorize as follows:

(a) To 50 ml of sample add measured quantity of saturated neutral Pb acetate soln, avoiding excess of Pb; filter, remove Pb with powdered anhydrous K oxalate, and filter. Polarize and correct for dilution with Pb acetate soln.

(b) To 50 ml of sample add decolorizing C, avoiding excessive amount or length of treatment. Filter thru double paper and polarize.

78

## SULFATES—OFFICIAL

To 100 ml of the absolutely clear sample, add 2 ml of approximately normal HCl; heat to boiling; add 10 ml of hot BaCl<sub>2</sub> soln (1 g per 100 ml), dropwise, and continue the boiling 5 min., keeping volume approximately constant by adding hot H<sub>2</sub>O from time to time as required. Allow mixture to stand until supernatant liquid is clear. (Overnight is convenient, but this time should not be exceeded.) Filter on ashless paper or weighed Munroe crucible.<sup>23</sup> Wash free from chlorides with hot H<sub>2</sub>O, dry, ignite at low red heat, cool, and weigh. Express result as mg of SO<sub>3</sub> in 100 ml of vinegar.

79

FORMIC ACID<sup>24</sup>—OFFICIAL

Use apparatus described under XXXII, 40. Introduce 100 ml of sample into flask (A); add 0.4–0.5 g of tartaric acid and place in position as shown in figure, having previously charged flask B with suspension of 15 g of CaCO<sub>3</sub> in 100 ml of H<sub>2</sub>O. Heat contents of flasks A and B to boiling and distil with steam from generator S, the vapor passing first thru sample in flask A, then thru the boiling suspension of CaCO<sub>3</sub> in flask B, after which it is condensed and measured in the receiver C. Maintain volume of liquid in flask B as nearly constant as possible and reduce volume of sample in flask A to 30–40 ml by heating with small Bunsen flames. Continue distillation until 1 liter of distillate is collected. Disconnect apparatus, filter the CaCO<sub>3</sub> suspension, and wash the CaCO<sub>3</sub> that remains on paper with a little hot H<sub>2</sub>O. Render filtrate faintly acid with HCl, add 10–15 ml of HgCl<sub>2</sub> reagent, XXXII, 39(b), mix, and heat on boiling water bath for 2 hours. Filter on weighed Gooch crucible and wash precipitate thoroly with cold H<sub>2</sub>O and finally with a little alcohol. Dry in boiling water oven for 30 min., cool in desiccator and weigh. Calculate weight of HCOOH present by multiplying weight of precipitate by 0.0975.

## TARTARIC ACID AND TARTRATES

80

*Qualitative Test—Official*

Evaporate 50 ml of sample in porcelain dish to volume of ca 10 ml, filter into test tube, add 1 ml of 25% CaCl<sub>2</sub> soln and 2 ml of 50% NH<sub>4</sub> acetate soln, and allow to stand overnight. In presence of tartaric acid a deposit of Ca tartrate is formed, the crystals of which may be identified under microscope by their characteristic form.

81

## TOTAL TARTARIC ACID—OFFICIAL

Evaporate 200 ml of sample to a sirupy consistency to remove excess of acetic acid, dilute to the original volume with H<sub>2</sub>O in a volumetric flask, determine the acidity as directed under 65, and determine total tartaric acid in a 100 ml aliquot as directed under XV, 28, using 20 ml of alcohol in the precipitation instead of 15 ml.

## FREE MINERAL ACIDS

82

*I. Logwood Method<sup>25</sup>—Tentative*

Prepare an extract of logwood as follows: Pour 100 ml of boiling H<sub>2</sub>O upon 2 g of fresh logwood chips, allow infusion to stand for a few hours, and filter. Place several drops of the liquid on porcelain surface and dry on water bath. Add to one of spots a drop of the vinegar to be tested (after concentration if desirable) and evaporate to dryness. A yellow tint remains if free mineral acids are absent, a red tint if they are present.

83

*II. Methyl Violet Method—Tentative*

Add 5–10 ml of H<sub>2</sub>O to 5 ml of vinegar, and after mixing well add 4 or 5 drops of methyl violet soln (1 part of methyl violet 2B in 10,000 parts of H<sub>2</sub>O). A blue or green coloration indicates presence of a free mineral acid.

84

*III. Quantitative Method—Tentative*

To a measured quantity of sample add a measured excess of standard alkali, evaporate to dryness, incinerate, and titrate ash with standard acid, using methyl orange indicator. Difference between number of ml of alkali first added and number of ml of acid needed to titrate ash = free mineral acid present.

85

## METALS—TENTATIVE.—See XXIX.

86

## DEXTRIN (QUALITATIVE TEST)—TENTATIVE

Evaporate 100 ml of the vinegar to a volume of ca 15 ml. Add slowly and with constant stirring 200 ml of alcohol and allow to stand overnight. Test precipitate formed by the optical rotation and color reaction with I.

## 87 SPICES AND ADDED PUNGENT MATERIALS (QUALITATIVE TEST)—TENTATIVE

Neutralize exactly a portion of the vinegar and test by taste and smell. Agitate liquid with ether in separatory funnel, remove, evaporate ethereal layer, and note odor and taste of residue.

88

## COLORING MATTERS—TENTATIVE.—See XXI.

89

## PRESERVATIVES—OFFICIAL.—See XXXII.

## SELECTED REFERENCES

- <sup>1</sup> Z. anal. Chem., **31**, 525 (1892); Conn. Agr. Exp. Sta. Rpt., 1898 (II), p. 190.
- <sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull., **13** (II) p. 165.
- <sup>3</sup> Conn. Agr. Exp. Sta. Rpt., 1898 (II), p. 187.
- <sup>4</sup> J. Assoc. Official Agr. Chem., **17**, 70, 371 (1934); **18**, 611 (1935).
- <sup>5</sup> Ibid., **18**, 83, 417 (1935).
- <sup>6</sup> Ibid., **19**, 98 (1936).
- <sup>7</sup> Ibid., **4**, 525 (1921).
- <sup>8</sup> Ibid., **11**, 523 (1928).
- <sup>9</sup> Winton, *Microscopy of Vegetable Foods*, 2nd ed., 1916; Vogl, *Die wichtigsten vegetabilischen Nahrungs und Genussmittel*, 1899; Tschirch and Oesterle, *Anatomischer Atlas der Pharmakognosie und Nahrungsmittelkunde*, 1900; Greenish and Collin, *Microscopical Examination of Foods and Drugs*, 2nd ed., 1910; Koch, *Die Mikroskopische Analyse der Drogenpulver*, 1900–08; Stephenson, *Some Microchemical Tests for Alkaloids*, 1921; Winton, *Structure and Composition of Foods*, 1932.
- <sup>10</sup> J. Am. Pharm. Assoc., **12**, 301 (1923).
- <sup>11</sup> J. Assoc. Official Agr. Chem., **7**, 71 (1923).
- <sup>12</sup> Ibid., **5**, 248 (1921); **7**, 138 (1923).
- <sup>13</sup> C. A. **10**, 3003 (1916).
- <sup>14</sup> J. Assoc. Official Agr. Chem., **16**, 77, 548 (1933).
- <sup>15</sup> Ibid., **20**, 527 (1937); **21**, 110 (1938); **22**, 607 (1939).
- <sup>16</sup> Ibid., **20**, 529 (1937).
- <sup>17</sup> Ibid., **8**, 150 (1924); **9**, 440 (1926); **10**, 490 (1927); **11**, 499 (1928).
- <sup>18</sup> J. Am. Chem. Soc., **22**, 218 (1900).
- <sup>19</sup> Ibid., **39**, 309 (1917); J. Ind. Eng. Chem., **5**, 845 (1913).
- <sup>20</sup> U. S. Dept. Agr. Bur. Chem. Bull., **137**, p. 61; J. Assoc. Official Agr. Chem., **3**, 411 (1920); **15**, 535 (1932); **18**, 82 (1935).
- <sup>21</sup> U. S. Dept. Agr. Bur. Chem. Bull. **105**, p. 23 (1907).
- <sup>22</sup> J. Assoc. Official Agr. Chem., **5**, 245 (1921); **8**, 151 (1924).
- <sup>23</sup> Z. anal. Chem., **2**, 241 (1888); J. Am. Chem. Soc., **31**, 456, 928 (1909).
- <sup>24</sup> Z. Nahr. Genussm., **21**, 1 (1911); **22**, 88 (1912).
- <sup>25</sup> Allen, *Commercial Organic Analysis*, 5th ed., 1923, Vol. 1, p. 666.

## XXXIV. SUGARS AND SUGAR PRODUCTS

### SUGARS, SIRUPS, AND MOLASSES

1

#### PREPARATION OF SAMPLE—OFFICIAL

(a) *Solids (sugars, etc.)*.—Grind, if necessary, and mix thoroly to secure uniform samples. In the case of raw sugars mix thoroly and in shortest possible time on glass plate with spatula, reducing lumps when present with glass or iron rolling pin; or mix thoroly and in shortest possible time in large, clean, dry mortar, using a pestle to reduce lumps when present.

(b) *Semi-solids (massecuites, etc.)*.—Weigh 50 g of sample, dissolve crystals of sugar in minimum amount of  $H_2O$ , wash into 250 ml volumetric flask, fill to mark, and mix thoroly; or weigh 50 g of sample and dilute with  $H_2O$  to 100 g. If insoluble material remains, mix uniformly by shaking before taking aliquots or weighed portions for various determinations.

(c) *Liquids (molasses, sirups, etc.)*.—Mix materials thoroly. If crystals of sugar are present, dissolve them either by heating gently (avoiding loss of  $H_2O$  by evaporation) or by weighing whole mass, then adding  $H_2O$ , heating until completely dissolved and after cooling, reweighing. Calculate all results to weight of original substance.

2

#### MOISTURE

##### *Direct Drying—Tentative*

Dry 2–5 g of prepared sample, 1(a), in flat dish (Ni, Pt, or Al) at temp. of boiling  $H_2O$  for 10 hours; cool in desiccator and weigh. Dry again for an hour or until change in weight is not more than 2 mg. In the case of sugars of large grain, heat at 105–110° to expel last traces of occluded  $H_2O$ .<sup>1</sup> Report loss in weight as moisture.

3

##### *Vacuum Drying Method—Official*

(Applicable to both cane and beet raw and refined sugars.)

Dry 2–5 g of the prepared sample, 1(a), in a flat dish of Ni, Pt, or Al, with a tight-fitting cover, at a temp. not exceeding 70° (preferably 60°), under a pressure not exceeding 50 mm of Hg, for 2 hours. Remove dish from oven, put cover in place, cool in desiccator, and weigh. Re-dry 1 hour and repeat process until change in weight between successive weighings at 1 hour intervals is not more than 2 mg. (Oven should be bled with a current of dry air during drying to insure removal of water vapors.)

4

##### *Drying upon Pumice Stone—Official*

(Applicable to massecuites, molasses, and other liquid and semiliquid products.)

Prepare pumice stone of 2 grades of fineness, one of which will pass thru 1 mm sieve, the other thru 6 mm but not 1 mm sieve. Digest each with  $H_2SO_4$  (1+4) for 8 hours on steam bath. Wash free from acid and heat to dull redness. Make the determination in flat metallic dish 60 mm in diameter. Place layer of the fine pumice stone, 3 mm in thickness, on bottom of dish, then a layer of the coarse pumice stone, 6–10 mm in thickness; dry, and weigh. Dilute sample with weighed portion of  $H_2O$  so that diluted material shall contain 20–30% of solid matter. Weigh into dish, prepared as described, quantity of diluted sample to yield ca 1 g of dry

matter. If this weighing can not be made rapidly, use weighing bottle provided with cork thru which pipet passes. Dry at 70° under pressure not to exceed 50 mm of Hg, making trial weighings at intervals of 2 hours toward end of drying period until change in weight does not exceed 2 mg. Report loss in weight as moisture. For substances containing little or no levulose or other readily decomposable substance, the drying may be made in water oven at temp. of boiling H<sub>2</sub>O.

5

*Drying upon Quartz Sand<sup>2</sup>—Official*

(Applicable to massecuites, molasses, and other liquid and semiliquid products.)

Digest pure quartz sand that will pass 40-mesh but not 60-mesh sieve with HCl, wash free from acid, dry, and ignite. Preserve in stoppered bottle. Place 25–30 g of prepared sand and short stirring rod in dish ca 55 mm in diameter and 40 mm in depth, fitted with cover. Dry thoroly, cover dish, cool in desiccator, and weigh immediately. Add sufficient diluted sample of known weight to yield ca 1 g of dry matter and mix thoroly with the sand. Heat on steam bath for 15–20 min., stirring at intervals of 2–3 min., or until mass becomes too stiff to manipulate readily. Dry at 70° under pressure of not to exceed 50 mm of Hg, making trial weighings at 2 hour intervals toward the end of drying period (ca 18 hours) until change in weight does not exceed 2 mg.

For materials containing no levulose or other readily decomposable substance dry at atmospheric pressure in water oven at temp. of boiling H<sub>2</sub>O, heat for 8–10 hours, cool in desiccator, and weigh, repeating the heating and weighing until loss in 1 hour does not exceed 2 mg. Report loss in weight as moisture.

As dry sand, as well as the dried sample, will absorb an appreciable quantity of moisture on standing over most desiccating agents, make all weighings as quickly as possible after cooling in desiccator.

SOLIDS

6

*By Means of a Spindle—Official*

(Not accurate when applied to low-grade sugar products, molasses, and other materials containing large quantities of non-sugar solids, but extensively used for approximate results.)

The density of juices, sirups, etc., is conveniently determined by means of the Brix or Baumé hydrometer, preferably the former, as the scale graduations agree closely with percentages of total solids. A table for comparison of degrees Brix (density scale indicating directly percentage by weight of pure sucrose in pure solns), degrees Baumé (modulus 145), sp. gr. at 20/4°, and sp. gr. at 20/20° is given under XLIII, 3.

Use spindle graduated in tenths, and as limited as possible in range of degrees recorded, and cylinder of sufficient diameter to permit spindle to come to rest without touching sides. Allow soln to come as nearly as practicable to same temp. as the air at time of reading, and if this varies more than 1° from temp. at which spindle was graduated, 20°, apply correction according to XLIII, 4. Before taking density of a juice, allow it to stand in cylinder until all air bubbles have escaped and all fatty or waxy matters have come to the top and been skimmed off. (Air bubbles may be conveniently removed, especially in case of solns of high density, by applying vacuum to the cylinder by means of a tube passing thru a stopper inserted in top of cylinder.) If sample is too dense to determine density directly, dilute weighed portion with a weighed quantity of H<sub>2</sub>O, or dissolve a weighed portion and dilute to known volume with H<sub>2</sub>O. In first instance the percentage of total solids is calculated by following formula:



Percentage of solids in undiluted material =  $\frac{WS}{w}$ , in which

$S$  = percentage of solids in diluted material;

$W$  = weight of diluted material; and

$w$  = weight of sample taken for dilution.

When dilution is made to definite volume, use following formula:

Percentage of solids in undiluted material =  $\frac{VDS}{w}$ , in which

$V$  = volume of diluted soln at given temp.;

$D$  = sp. gr. of diluted soln at same temp.;

$S$  = percentage of solids in diluted soln at same temp.; and

$w$  = weight of sample taken for dilution.

*Double dilution.*—The calculation is simplified by mixing equal weights of sugar product and water, and multiplying the Brix of the soln by two.

## 7

*By Means of a Pycnometer<sup>3</sup>—Official*

(a) *Specific gravity (in vacuo or in air).*—Determine sp. gr. of soln at 20/4°, 20/20° in vacuo, or 20/20° in air as directed under XIV, 3, and ascertain corresponding percentage by weight of sugar solids from appropriate table, XLIII, 3, or 5. When density of substance is too high for direct determination, dilute and then calculate sucrose content of original material as directed under 6.

(b) *Specific gravity of molasses.*—Use a special 100 ml volumetric flask with neck ca 8 mm inside diameter. Weigh flask empty and then fill it with molasses, using long-stemmed funnel reaching below graduation mark, until level of molasses is up to lower end of neck of flask. (The flow of molasses may be stopped by inserting glass rod of suitable size into funnel so as to close stem opening.) Remove funnel carefully to prevent molasses coming in contact with the neck, and weigh flask and molasses. Add H<sub>2</sub>O almost to graduation mark, running it down side of neck to prevent mixing with the molasses. Allow to stand several hours or overnight to permit escape of bubbles. Place flask in constant temp. H<sub>2</sub>O bath, preferably at 20°, and leave until it reaches temp. of bath; then make to volume at that temp. with H<sub>2</sub>O. Weigh. Reduce weight of molasses to vacuo and calculate density. Ascertain corresponding Brix or Baumé reading from XLIII, Table 3.

*Example:*

	<i>grams</i>
A, wt H <sub>2</sub> O content flask 20° in vacuo	= 99.823
B, wt molasses 20° in vacuo	= 132.834
C, wt molasses and H <sub>2</sub> O 20° in vacuo	= 137.968
A - (C - B) = wt H <sub>2</sub> O occupying space of molasses in vacuo	= 94.689

$$\frac{132.834}{94.689} = 1.403 \text{ sp. gr. } \left( \frac{20^\circ}{20^\circ} \right) \text{ molasses.}$$

## 8

*By Means of Refractometer<sup>4</sup>—Official*

(Applicable only to liquid samples containing no undissolved solids.)

Determine refractometer reading of soln at 20° and obtain corresponding percentage of dry substance from either the direct reading, if a sugar refractometer is used, or from Table 6, XLIII, if the instrument gives readings in terms of refractive index. Circulate H<sub>2</sub>O at constant temp., preferably 20°, thru jackets of refractom-

eter or thru trough of immersion instrument, long enough to allow temp. of prisms and of sample to reach equilibrium, continuing circulation during observations and taking care that constant temp. is maintained. If the determination is made at a temp. other than 20°, or if humidity causes condensation of moisture on exposed faces of the prisms, make measurements at room temp. and correct readings to standard temp. of 20° according to Table 7, XLIII. If soln is too dark to be read in the instrument, dilute with a concentrated sugar soln; never use H<sub>2</sub>O for this purpose. Mix weighed quantities of soln under examination and a soln of pure sugar of about same strength, and obtain the quantity of dry substance in the former by following formula:

$$x = \frac{(A+B)C - BD}{A}, \text{ in which}$$

$x$  = percentage of dry substance to be found;

$A$  = weight (g) of sample mixed with  $B$ ;

$B$  = weight (g) of pure sugar soln used in dilution;

$C$  = percentage of dry substance in mixture  $A+B$  obtained from the refractive index; and

$D$  = percentage of dry substance in the pure sugar soln obtained from its refractive index.

#### ASH—OFFICIAL

9

##### *Method I.*

Heat 5–10 g of sample in 50–100 ml Pt dish at 100° until H<sub>2</sub>O is expelled, add few drops of pure olive oil, and heat slowly over flame until swelling ceases. Place dish in muffle at ca 525° and leave until a white ash is obtained. Moisten ash with H<sub>2</sub>O, dry on steam bath and then on hot plate, and re-ash in muffle at 525° to constant weight.

10

##### *Method II.*

Carbonize 5–10 g of sample in 50–100 ml Pt dish at ca 525° and treat charred mass with hot H<sub>2</sub>O to dissolve soluble salts. (In case of low-purity products the addition of a few drops of pure olive oil, as in 9, may be desirable.) Filter thru ashless filter, ignite filter and residue to white ash, add filtrate of soluble salts, evaporate to dryness, and ignite at ca 525° to constant weight.

11

##### *Sulfated Ash*

Weigh 5 g of sample into 50–100 ml Pt dish, add 5 ml of 10% H<sub>2</sub>SO<sub>4</sub>, ignite until sample is well carbonized, and then burn in muffle at ca 550°. Cool, add 2–3 ml of 10% H<sub>2</sub>SO<sub>4</sub>, evaporate on steam bath, dry on hot plate, and again ignite at 550° to constant weight. Express result as percentage of sulfated ash.

12

#### MINERAL CONSTITUENTS—OFFICIAL.—See XII.

13

#### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Ash the material as directed, 9 or 10. Add H<sub>2</sub>O to ash in the Pt dish, heat nearly to boiling, filter thru ashless filter, and wash with hot H<sub>2</sub>O until combined filtrate and washings measure ca 60 ml. Return filter and contents to Pt dish, ignite carefully, cool, and weigh. Calculate percentages of water-soluble and water-insoluble ash.

14

#### ALKALINITY OF SOLUBLE ASH—OFFICIAL

Cool filtrate from 13 and titrate with 0.1 N HCl, XLII, 5 and 6, using methyl or-

ange indicator, VI, 3(f). Express alkalinity in terms of number of ml of normal acid per 100 g of sample.

15

## ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Add excess of 0.1 N HCl (usually 10–15 ml) to ignited insoluble ash in Pt dish, 13, heat to incipient boiling on asbestos plate, cool, and titrate excess of HCl with 0.1 N NaOH, using methyl orange indicator. Express alkalinity in terms of number of ml of normal acid per 100 g of sample.

16

MINERAL ADULTERANTS IN THE ASH<sup>5</sup>—TENTATIVE

In large porcelain evaporating dish, mix 100 g of sample with ca 35 g of H<sub>2</sub>SO<sub>4</sub> and evaporate to sirupy consistency. Pass electric current thru it while stirring by placing one Pt electrode in bottom of dish near one side and attaching other to lower end of the glass rod with which contents are stirred. Begin with current of ca 1 ampere and gradually increase to 4. In 10–15 min. the mass is reduced to fine dry char, which may be readily burned to white ash in original dish over free flame or in muffle.

This method is preferred to ordinary method of heating with H<sub>2</sub>SO<sub>4</sub>, especially in the case of molasses, because if properly manipulated the material comes quickly into form of very finely divided char or powder that is especially adapted for subsequent quick ignition.

If electric current is not available, treat in large porcelain dish 100 g of the saccharine soln, evaporate to sirupy consistency with sufficient H<sub>2</sub>SO<sub>4</sub> to carbonize mass thoroly, and ignite in usual manner.

The following adulterants may be present: salts of Sn, used in molasses to bleach; mineral pigments, such as PbCrO<sub>4</sub> in yellow confectionery; oxide of iron, sometimes used to simulate color of chocolate, and Cu. These elements may be detected by the usual qualitative tests.

17

## NITROGEN—OFFICIAL

Determine N in 5 g of the material as directed under II, 21, 22, or 23, using larger quantity of the H<sub>2</sub>SO<sub>4</sub> if necessary for complete digestion.

## SUCROSE—POLARIMETRIC METHODS

## GENERAL PROCEDURE

18

(a) *Directions for Raw Sugars—Official*

(Rules of International Commission for Unifying Methods of Sugar Analysis.<sup>6</sup>)

"In general all polarizations are to be made at 20°.

"The verification of the saccharimeter must also be made at 20°. For instruments using the Ventzke scale 26 g of pure dry sucrose, weighed in air with brass weights, dissolved in H<sub>2</sub>O so that 100 ml of soln is obtained at 20° and polarized in a room or cabinet, the temp. of which is also 20°, must give a saccharimeter reading of exactly 100.00. The temp. of the sugar soln during polarization must be kept constant at 20°."

According to the determination of Bates and Jackson<sup>7</sup> at National Bureau of Standards, the Ventzke scale saccharimeter reading for 26 g of pure dry sucrose under the above conditions is 99.895. The Ventzke scale reading has been redetermined by Balch and Hill<sup>8</sup> and by Zerban, Gamble, and Hardin,<sup>9</sup> who found values of 99.907° and 99.912°, respectively. The average value of these three independent observers is 99.90.

"For countries where the mean temp. is higher than 20°, saccharimeters may be adjusted at 30° or any other suitable temp., under conditions specified above, provided the sugar soln be diluted to final volume and polarized at this same temp.

"In determining the polarization of substances containing sugar employ only half-shade instruments."

The saccharimeter used may have either a single or double wedge and should be a half-shadow instrument with either double or triple field.

"During the observation keep the apparatus in a fixed position and so far removed from the source of light that the polarizing Nicol is not warmed.

"As sources of light, employ lamps which give a strong illumination, such as triple gas burner with metallic cylinder, lens, and reflector; gas lamps with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; or Na light. Whenever there is any irregularity in the sources of light such as that due to the convolutions of the filament in the case of electric light or to the meshes of the gauze in the case of the Welsbach light, place a thin ground-glass plate between the source of light and the polariscope so as to render the illumination uniform.

"Before and after each set of observations the chemist must satisfy himself of the correct adjustment of his saccharimeter by means of standardized quartz plates. He must also previously satisfy himself of the accuracy of his weights, polarization flasks, observation tubes, and cover-glasses. (Scratched cover-glasses must not be used.) Make several readings and take the mean thereof, but no one reading may be neglected."

The quartz plates are standardized to read to the second decimal place, and by their use a quick and at the same time accurate test can be made. In using such plates for testing saccharimeters, it is necessary that the instrument, as well as the plate, be at 20° before a reading is made. Different points of the scale, preferably 20°, 50°, 80°, and 100° (sugar scale), should be tested against the plates. The scale may also be standardized by means of a carefully calibrated telescopic control tube.<sup>9</sup> A new type of telescopic control tube of high accuracy has recently been described by Zerban, Gamble, and Hardin.<sup>8</sup>

"In determining the polarization use the whole normal weight for 100 ml or a multiple thereof for any corresponding volume.

"As clarifying and decolorizing agents use basic acetate of lead . . . , alumina cream, or concentrated soln of alum. Boneblack and decolorizing powders are to be excluded."

Whenever reducing sugars are determined in the soln for polarizing, use only neutral Pb acetate for clarification, as basic Pb acetate causes precipitation of some of the reducing sugars. In addition to the clarifying agents mentioned, basic Pb nitrate has been made official by the Association.

"After bringing the soln exactly to the mark at the proper temp., and after wiping out the neck of the flask with filter paper, pour all the well-shaken clarified sugar soln on a rapidly acting, dry filter. Reject the first portion of the filtrate and use the remainder, which must be perfectly clear, for polarization."

Cover the funnel at the start of filtration. It is advisable to reject the first 25 ml that runs thru, and use remainder for polarization. In no case should whole soln or any part be returned to filter. If cloudy after the 25 ml has been rejected, begin a new determination.

"Whenever white light is used in polarimetric determinations, the same must be filtered thru a soln of  $K_2Cr_2O_7$  of such a concentration that the percentage content of the soln multiplied by the length of the column of the soln in centimeters is equal to nine."

This concentration must be doubled in polarizing carbohydrate materials of high rotation dispersion, such as commercial glucose, etc.

(b) *Conversion Factors of Different Saccharimeter Scales*

1 Ventzke Scale	=0.34657° Angular Rotation D
1° Angular Rotation D*	=2.88542° Ventzke Scale
Normal weight Ventzke Scale	=26.026 grams
1° Bureau Standards Scale	=0.34620° Angular Rotation D
1° Angular Rotation D	=2.88550° Bureau Standards Scale
Normal weight Bur. Stand. Scale	=26.000 grams
1° Bidecimal Scale	=0.26622° Angular Rotation D
1° Angular Rotation D	=3.75629° Bidecimal Scale
Normal weight Bidecimal Scale	=20.000 grams
1° French Scale	=0.21667° Angular Rotation D
1° Angular Rotation D	=4.61538° French Scale
Normal weight French Scale	=16.27 grams

19

*Preparation and Use of Clarifying Reagents<sup>10</sup>—Official*

(a) *Basic lead acetate soln.*—Boil 430 g of neutral Pb acetate, 130 g of litharge, and 1 liter of H<sub>2</sub>O for 30 min. Allow mixture to cool and settle and then dilute the supernatant liquid to a sp. gr. of 1.25 with recently boiled H<sub>2</sub>O. Solid basic Pb acetate may be substituted for the normal salt and litharge in the preparation of the soln. (This reagent is used primarily for clarifying dark colored cane, sorghum, and beet products when sucrose is determined by polariscopic methods.)

(b) *Alumina cream.*—Prepare a cold saturated soln of alum in H<sub>2</sub>O. Add NH<sub>4</sub>OH with constant stirring until the soln is alkaline to litmus, allow precipitate to settle, and wash by decantation with H<sub>2</sub>O until the wash H<sub>2</sub>O gives only a slight test for sulfates with BaCl<sub>2</sub> soln. Pour off the excess of H<sub>2</sub>O and store the residual cream in stoppered bottle. (Alumina cream is suitable for clarifying light colored sugar products or as an adjunct to other agents when sugars are determined by polariscopic or reducing sugar methods.)

(c) *Dry basic lead acetate.*—Obtained as a dry powdered salt and should contain 72.8% of Pb, which corresponds to a composition of 3 Pb(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub> · 2 PbO. Of this salt, ca  $\frac{1}{3}$  g = 1 ml of the basic Pb acetate soln described under (a). In making the clarification, add a small quantity of the dry salt to the sugar soln after completion to volume and shake; then add more salt and shake again, repeating the addition until precipitation is complete, but avoiding any excess. When molasses or any other substance producing a heavy precipitate is being clarified, add some dry, coarse sand to break up the pellets of basic Pb acetate and precipitate. (Dry basic Pb acetate can also be used in place of soln of this salt in clarifying cane, sorghum, and beet products.)

(d) *Neutral lead acetate.*—Prepare a saturated soln of neutral Pb acetate and add it to the sugar soln before completing to volume. (This reagent may be used for clarifying light-colored sugar products when sugars are determined by polariscopic methods, and its use is imperative when reducing sugars are determined in the soln used for polarization.)

(e) *Basic lead nitrate.*—(1) Dissolve 250 g of Pb(NO<sub>3</sub>)<sub>2</sub> in H<sub>2</sub>O and make up to 500 ml. (2) Dissolve 25 g of NaOH in H<sub>2</sub>O and make up to 500 ml. In making the

\* The designation D refers to sodium light of 5893° Ångström.

clarification, add equal quantities of (1) and (2) to the sugar soln, shake, and add more if complete precipitation has not occurred, but avoid an excess. Complete the volume with  $H_2O$ . (This reagent is used for the same purposes as the one described under 19(a).) When this soln is used for clarification, the basic value of the divisor in the Clerget determination by acid becomes 143.5.

## 20 *Temperature Corrections for the Polarization of Sugars*<sup>11</sup>—*Tentative*

(a) *Refined sugars*.—The polarizations of sugars testing 99 or above, when made at a temp. other than 20°, may be calculated to polarizations at 20° by the following formula:

$$P_{20} = p^t[(1 + 0.0003(t - 20))], \text{ in which}$$

$$p^t = \text{polarization at temp. read; and}$$

$$t = \text{temp. at which polarization is read.}$$

(b) *Raw cane sugars*.—The polarizations of raw cane sugars, when made at temps. other than 20°, may be calculated to polarizations at 20° by the following formula:

$$P_{20} = p^t + 0.0015(p^t - 80)(t - 20), \text{ in which}$$

$$p^t \text{ and } t \text{ are the same as in the formula under (a).}$$

When the percentage of levulose in the sugar is known (which in the case of honeys and sugar cane products is ca  $\frac{1}{2}$  the reducing sugars), the following formula should be used:

$$P_{20} = p^t + 0.0003S(t - 20) - 0.00812L(t - 20), \text{ in which}$$

$$p^t \text{ and } t \text{ are the same as in the formula under (a);}$$

$$S = \text{percentage of sucrose; and}$$

$$L = \text{percentage of levulose.}$$

These formulas give results agreeing closely with the polarizations obtained at 20° if the sugar is of average normal composition.

## 21 *Mutarotation*

Products, such as honey and commercial glucose, that contain dextrose or other reducing sugars in crystalline form or in soln at high density may show mutarotation under the conditions prevailing during analysis. Only constant rotation should be used in polarimetric methods. To obtain this, allow the soln prepared for polarization to stand overnight before making reading. If it is desired to make reading immediately, heat the neutral (pH ca 7.0) soln to boiling for a few moments or add a few drops of  $NH_4OH$  before completing to volume; or, if soln has been made to volume, add dry  $Na_2CO_3$  until just distinctly alkaline to litmus paper. (Do not allow the slightly alkaline solns to stand at such high temps. or for such lengths of time as to cause destruction of fructose.) Determine completion of mutarotation by making readings at 15–30 min. intervals until these become constant.

### SUCROSE IN THE ABSENCE OF RAFFINOSE

#### *I. By Polarization Before and After Inversion with Invertase*<sup>12</sup>—*Official*

## 22

### REAGENT

*Invertase soln*.—Commercial invertase preparations are available on the market. If it is desired to prepare the soln in the laboratory, the procedure described under (1) may be used. In either case the preparation may be further purified and concentrated by the ultrafiltration method described under (3). Commercial preparations may also be purified by dialysis and then reconcentrated by evaporating in vacuo at a temp. not to exceed 40°.

(1) *Crude invertase soln.*—Mix yeast with  $H_2O$  in the proportion of 10 lbs of compressed bakers' yeast to 5 liters of  $H_2O$ . Add 2 liters of toluene and stir thoroly at frequent intervals during the first 24 hours. Allow to stand for 7 days with occasional stirring and filter by gravity thru large fluted papers. Mix the residue with 2 liters of  $H_2O$ , filter, and combine the filtrates. Purify<sup>13</sup> by adding 15 g of neutral Pb acetate to each liter of extract and filtering on paper after all Pb acetate has been dissolved. Complete the purification immediately by dialysis or by washing on the ultrafilter as directed under (3).

(2) *Collodion ultrafilter.*<sup>14</sup>—Dissolve 6 g of soluble (in alcohol and ether mixture) pyroxylin or nitro-cellulose such as Astoria's in a mixture of 50 ml of absolute alcohol and 50 ml of absolute ether by first adding the alcohol to the cotton, allowing the mixture to stand in a stoppered flask for 10 min., adding the ether, and shaking. Allow soln to stand overnight, pour ca 100 ml into 2000 ml cylinder, and coat entire inside surface of cylinder with the collodion. Drain, and dry for 10 min. Fill with  $H_2O$ , let stand 10–15 min., pour out the  $H_2O$ , and remove the collodion sack. Test for leaks by filling with  $H_2O$ . Slit open longitudinally and cut out a circular piece ca 7–8" in diameter. Cut the bottom from 2 liter bottle or Erlenmeyer flask and grind edge smooth. Place it upon the still moist collodion disk, fold edge of disk up around bottle, and cement it thereto with collodion that contains an increased percentage of ether. Place 3 or 4 thicknesses of wet filter paper in an 8" Büchner funnel. Place the bottle with the collodion membrane upon the filter paper. Pour melted vaseline, to depth of an inch, between bottle and inside of funnel. Provide bottle with a small mechanical stirring device.

(3) *Washing and concentration of invertase soln by ultrafiltration.*—Filter 4 liters of the partially purified soln thru the ultrafilter, stirring continuously, until about 1 liter remains. Wash with distilled  $H_2O$  introduced by means of a constant level device until the filtrate is colorless, 3 or 4 liters of wash  $H_2O$  being required. During the entire process the invertase soln should be preserved with toluene.

(4) *Activity of invertase soln.*—The following test for activity of the invertase soln is usually adequate: Dilute 1 ml of the invertase preparation to 200 ml. Transfer 10 g of sucrose (granulated sugar) to a sugar flask graduated at 100 ml and 110 ml, dissolve in ca 75 ml of  $H_2O$ , add 2 drops of glacial acetic acid, and dilute to the 100 ml mark. To the 100 ml of sugar soln add 10 ml of the dilute invertase soln and mix thoroly and rapidly, noting exact time at which solns are mixed. At the termination of exactly 60 min. make a portion of the soln just distinctly alkaline to litmus paper with anhydrous  $Na_2CO_3$  and polarize in 200 mm tube at 20°. If the invertase soln is sufficiently active, the alkaline soln will polarize approximately 31° Ventzke without correcting for the dilution to 110 ml and the optical activity of the invertase soln.

If more exact information concerning the activity of the invertase preparation is desired, determine its velocity constant as follows: Dilute 1 ml of the invertase soln to 200 ml at 20°; place in constant temp. bath at 20°; and when soln has attained the latter temp., pipet 20 ml of it into a flask containing 200 ml of a sucrose soln (10 g per 100 ml concentration) that has been previously made distinctly acid to methyl red (corresponding to pH ca 4.6<sup>15</sup>) by addition of acetic acid and also brought to temp. of 20° in same bath. Mix thoroly and promptly and note time at which invertase soln was added. Keep sucrose-invertase mixture in constant temp. bath; remove portions at end of 15, 30, and 45 min.; render each portion just distinctly alkaline to litmus paper with anhydrous  $Na_2CO_3$  immediately after removing; and polarize at 20°. Correct all polarizations for the polarization of the invertase soln. Calculate velocity constant,  $k$ , for each of polarizations (at the time  $t$ ) subsequent to the initial polarization by the following formula:

$$k = \frac{\log_{10} 1.32 R_0 - \log_{10} (R_t + 0.32 R_0)}{t}, \text{ in which}$$

$k$  = unimolecular reaction velocity constant;

$t$  = number of min. elapsing from time invertase and sucrose solns were mixed until inversion was stopped by addition of  $\text{Na}_2\text{CO}_3$ ;

$R_0$  = initial polarization (calculated by multiplying polarization of sucrose soln by 10/11 and correcting for polarization of invertase soln); and

$R_t$  = polarization at time  $t$ .

An invertase soln of sufficient activity <sup>16</sup> should yield an average value for  $k$  (for various time periods) of at least 0.1 after multiplying the  $k$  value directly obtained by 200, in order to correct for initial dilution of invertase soln. Dilution of invertase soln above mentioned is made solely for purpose of determining its activity; the original, undiluted invertase soln is used as the inverting reagent in determination of sucrose (23) unless the activity of the original invertase soln greatly exceeds a  $k$  value of 0.1, and it is desirable to conserve the invertase. In this case, dilute to  $k$  value of 0.1, which is done in same manner as diluting other solns to standard strength. The activity of the invertase preparation required for rapid inversion, 23(c), is the same as that needed for overnight inversion, 23(b), but the proportion of invertase preparation used in the former case is twice that used in the latter instance.

## 23

## DETERMINATION

(a) *Direct reading*.—Dissolve the double normal weight of the substance (52 g), or a fraction thereof, in  $\text{H}_2\text{O}$  in 200 ml volumetric flask; add necessary clarifying agent, 19(a), (b), (d), or (e), avoiding any excess; shake, dilute to mark with  $\text{H}_2\text{O}$ , mix well, and filter, keeping funnel covered with watch-glass. Reject first 25 ml of filtrate. If a Pb clarifying agent was used, remove excess Pb from soln when sufficient filtrate has collected by adding anhydrous  $\text{Na}_2\text{CO}_3$ , a little at a time, avoiding any excess; mix well and filter again, rejecting first 25 ml of filtrate. (Instead of weighing 52 g into 200 ml flask, two 26 g portions may be diluted to 100 ml each, and treated exactly as described. Depending on color of the product, multiples or fractions of the normal weight may be used, and results reduced by calculation to basis of 26 g in 100 ml.) Pipet one 50 ml portion of the Pb-free filtrate into 100 ml flask, dilute with  $\text{H}_2\text{O}$  to mark, mix well, and polarize in 200 mm tube. The result, multiplied by 2, is direct reading ( $P$  of formula given below) or polarization before inversion. (If 400 mm tube is used, the reading equals  $P$ .) If there is a possibility of mutarotation, proceed as directed under 21.

(b) *Invert reading*.—First determine quantity of acetic acid necessary to render 50 ml of the Pb-free filtrate distinctly acid to methyl red indicator; then to another 50 ml of the Pb-free soln in 100 ml volumetric flask, add requisite quantity of acid and 5 ml of the invertase preparation, fill flask with  $\text{H}_2\text{O}$  nearly to 100 ml, and let stand overnight (preferably at not less than  $20^\circ$ ). Cool, and dilute to 100 ml at  $20^\circ$ . Mix well and polarize at  $20^\circ$  in 200 mm tube. If in doubt as to completion of hydrolysis, allow a portion of soln to remain for several hours and again polarize. If there is no change from previous reading, inversion is complete. Carefully note reading and temp. of the soln. If it is necessary to work at a temp. other than  $20^\circ$ , which is permissible within narrow limits, complete volumes and make both direct and invert readings at same temp. Correct polarization for the optical activity of the invertase soln and multiply by 2. Calculate percentage of sucrose by following formula:



$$S = \frac{100 (P - I)}{142.1 + 0.073 (m - 13) - t/2}, \text{ in which}$$

$S$  = percentage of sucrose;

$P$  = direct reading, normal soln;

$I$  = invert reading, normal soln;

$t$  = temp. at which readings are made; and

$m$  = g of total solids from original sample in 100 ml of the invert soln.

Determine total solids as percentage by weight, as directed under 8, and multiply this figure by density at 20°, XLIII, 3.

(c) *Rapid inversion at 55–60°*.<sup>17</sup>—If more rapid inversion is desired, proceed as follows: Prepare sample as directed under (a) and to 50 ml of the Pb-free filtrate in a 100 ml volumetric flask add glacial acetic acid in sufficient quantity to render soln distinctly acid to methyl red, 22(4). The quantity of acetic acid required should be determined before pipetting the 50 ml portion as directed under 23(b). Add 10 ml of invertase soln, mix thoroly, place flask in water bath at 55–60°, and allow to stand at that temp. for 15 min. with occasional shaking. Cool, add Na<sub>2</sub>CO<sub>3</sub> until distinctly alkaline to litmus paper, dilute to 100 ml at 20°, mix well, and determine the polarization at 20° in 200 mm tube. Allow soln to remain in tube for 10 min. and again determine polarization. If there is no change from previous reading, mutarotation is complete. Carefully note reading and temp. of the soln. Correct polarization for optical activity of the invertase soln and multiply by 2. Calculate percentage of sucrose by formula given under (b).

If the soln has been rendered so alkaline as to cause destruction of sugar, the polarization, if negative, will in general decrease, since the decomposition of fructose ordinarily is more rapid than that of the other sugars present. If the soln has not been made sufficiently alkaline to complete mutarotation quickly, the polarization, if negative, will in general increase. As the analyst gains experience he may omit the polarization after 10 min. if he has satisfied himself that he is adding Na<sub>2</sub>CO<sub>3</sub> in sufficient amount to complete mutarotation at once without causing any destruction of sugar during the period intervening before polarization.

## 24 II. By Polarization Before and After Inversion with Hydrochloric Acid<sup>18</sup>—Official

(In the presence of much levulose, as in honeys, fruit products, sorghum sirup, cane sirup, and molasses, the optical method for sucrose, requiring hydrolysis by acid, gives erroneous results.)

(a) *Direct reading*.—Proceed as directed under 23(a).

(b) *Invert reading*.—Pipet a 50 ml portion of the Pb-free filtrate into a 100 ml flask and add 25 ml of H<sub>2</sub>O. Add, little by little, while rotating flask, 10 ml of HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°). Heat water bath to 70° and regulate burner so that temp. of bath remains approximately at that point. Place flask in water bath, insert thermometer, and heat with constant agitation until thermometer in flask indicates 67°. (This preliminary heating period should require 2½–2¾ min.) From the moment the thermometer in flask indicates 67°, leave flask in bath exactly 5 min. longer, during which time the temp. should gradually rise to ca 69.5°. Plunge flask at once into H<sub>2</sub>O at 20°. When contents have cooled to ca 35°, remove thermometer from flask, rinse it, and fill almost to mark. Leave flask in bath at 20° at least 30 min. longer and finally make up exactly to volume. Mix well and polarize soln in 200 mm tube provided with a lateral branch and water jacket, maintaining temp. of 20°. This reading must also be multiplied by 2 to obtain the invert reading. If it is necessary to work at a temp. other than 20°, which is permissible within nar-

row limits, the volumes must be completed and both direct and invert polarizations must be made at exactly the same temp.

Calculate sucrose by following formula:

$$S = \frac{100 (P - I)}{143 + 0.0676 (m - 13) - t/2}, \text{ in which}$$

$S$  = percentage of sucrose;

$P$  = direct reading, normal soln;

$I$  = invert reading, normal soln;

$t$  = temp. at which readings are made; and

$m$  = g of total solids from original sample in 100 ml of invert soln.

Determine total solids as percentage by weight, as directed under 8, and multiply this figure by the density at 20°, XLIII, 3.

(c) *Inversion at room temperature.*—The inversion may also be accomplished as follows: (1) To 50 ml of the clarified soln, freed from Pb, add 10 ml of HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°) and set aside for 24 hours at a temp. not below 20°; or, (2) if temp. is above 25°, set aside for 10 hours. Make up to 100 ml at 20° and polarize as directed under (b). Under these conditions the formula must be changed to the following:

$$S = \frac{100 (P - I)}{143.2 + 0.0676 (m - 13) - t/2}.$$

#### SUCROSE AND RAFFINOSE<sup>19</sup>

##### *I. By Polarization Before and After Treatment with Two Enzyme Preparations—Official*

#### 25

##### REAGENTS

(a) *Invertase soln (top yeast extract).*—Prepare as directed under 22. This soln should be free from the enzyme melibiase. Its invertase activity should be at least as great as that used for determination of sucrose in the absence of raffinose, 22(4).

(b) *Invertase-melibiase soln (bottom yeast extract).*—Prepare as directed under 22, using bottom fermenting yeast (brewers' yeast) instead of bakers' yeast. The invertase activity should be at least as great as in (a).

Test the melibiase activity of the soln as follows: Add 2 ml of the soln to be tested to 20 ml of a weakly acid melibiose soln polarizing +20.0°V and allow to stand 30 min. at ca 20°. Add sufficient Na<sub>2</sub>CO<sub>3</sub> to render soln slightly alkaline to litmus paper. A preparation suitable for the overnight hydrolysis of solns containing not more than 0.2 g of raffinose in 100 ml should have hydrolyzed 35% of the melibiose present under conditions mentioned; a preparation suitable for overnight hydrolysis of solns containing not more than 0.65 g of raffinose in 100 ml should have produced 50% hydrolysis of melibiose; and a preparation suitable for overnight hydrolysis of solns containing 0.65–1.3 g of raffinose in 100 ml should have hydrolyzed at least 70% of the melibiose present under above condition. The polarizations that correspond to 35, 50 and 70% hydrolysis of a melibiose soln polarizing, before hydrolysis, +20°V are: +16.4°, +14.9° and +12.9°V, respectively.

#### 26

##### DETERMINATION

In analyzing sugar beet products, weigh the quantity of material specified in the following table, transfer to 300 ml volumetric flask, add quantity of basic Pb acetate soln indicated in table, and dilute to volume at 20°. Mix thoroly and filter thru fluted paper in a closely covered funnel, rejecting first 25 ml of filtrate. When

*Quantities of sample and reagents required for clarification and deleading of beet sugar-house products*

MATERIAL	QUANTITY PER 100 ML	BASIC LEAD ACETATE (55° BRIX)	AMMONIUM DIHYDROGEN PHOSPHATE
	<i>grams</i>	<i>ml</i>	<i>gram</i>
Cossettes <sup>a</sup> .....	13	3	0.2
Pulp.....	100 ml <sup>b</sup>	2-4	0.2
Lime cake or sewer <sup>c</sup> .....	26.5	1.5	..... <sup>d</sup>
Thin juice.....	52	2	0.2-0.3
Thick juice.....	26	4	0.3-0.4
White massecuite.....	13 or 26	3 or 6	0.3-0.7
High wash sirup.....	13 or 26	3 or 6	0.3-0.7
High green sirup.....	13 or 26	5 or 10	0.3-0.7
Raw or remelt massecuite.....	13	6	0.3-0.4
Raw or remelt sugar.....	26	3-4	0.3-0.4
Sugar melter.....	26	2-3	0.3-0.4
Low wash sirup.....	13	8-10	0.4-0.5
Low green sirup or molasses.....	13	10	0.4-0.5
Saccharate cakes and milk (carbonated)	26	4-6	0.3-0.4
Steffen waste and wash waters <sup>c</sup> .....	78 or 50 ml	2-3	0.2

<sup>a</sup> Usual method of extraction, 26 g in 201.2 ml.

<sup>b</sup> Dilute to 110 ml.

<sup>c</sup> Neutralize with acetic acid before adding basic Pb acetate.

<sup>d</sup> Lime in soln will be partly precipitated by the phosphate, and it is necessary to add sufficient phosphate to complete the precipitation of both the lead and lime salts; hence no definite quantity can be specified.

sufficient filtrate has collected, remove Pb from soln by adding  $\text{NH}_4\text{H}_2\text{PO}_4$  in as small excess as possible (see table). This condition is readily determined after a little practice by appearance of the  $\text{Pb}_3(\text{PO}_4)_2$  precipitate, which usually flocculates and settles rapidly in presence of a slight excess of the salt. Mix well and filter, again rejecting at least the first 25 ml of filtrate. Make a direct polarization in 200 mm tube at 20° unless soln contains an appreciable quantity of invert sugar, in which case pipet a 50 ml portion of the Pb-free filtrate into 100 ml flask, dilute with  $\text{H}_2\text{O}$  to mark, mix well, and polarize at 20°, preferably in 400 mm tube. This reading, calculated to normal weight of 26 g in 100 ml and 200 mm tube length, is the direct reading ( $P$ ) of formula given below for polarization before inversion.

Transfer two 50 ml portions of the Pb-free filtrate to 100 ml flasks. To one add 5 ml of invertase soln (top yeast extract) and to the other 5 ml of invertase-melibiose soln (bottom yeast extract), let stand overnight at atmospheric temp. (preferably not below 20°), dilute to volume, mix well, and polarize at 20°, preferably in 400 mm jacketed tube. If rapid hydrolysis is desired, add 10 ml of each of the enzyme solns to the 50 ml portions of deleading filtrate in 100 ml flasks and place in water bath at 50-55° for 40 min. Then add  $\text{Na}_2\text{CO}_3$  until soln is slightly alkaline to litmus paper, dilute to volume at 20°, mix well, and polarize at 20°, preferably in 400 mm tube. Correct invert readings for the optical activity of the enzyme soln and calculate polarization to that of a normal weight soln of 26 g in 100 ml; also calculate reading to 200 mm tube length, if necessary.

Calculate percentages of anhydrous raffinose and sucrose from following formulas:

$$R = 1.354 (A - B);$$

$$S = \frac{(P - 2.202A + 1.202B)100}{132.12 - 0.00718[132.12 - (P - 2.202A + 1.202B)]}, \text{ in which}$$

$R$  = percentage of raffinose;

$S$  = percentage of sucrose;

$P$  = direct polarization, normal soln;

$A$  = corrected polarization after top yeast hydrolysis, normal soln; and

$B$  = corrected polarization after bottom yeast hydrolysis, normal soln.

The quantities  $A$  and  $B$  are treated algebraically.

## 27 *II. By Polarization Before and After Inversion with HCl—Official*

(Of value chiefly in analysis of beet products.)

If the direct reading is more than  $1^\circ$  higher than the percentage of sucrose as calculated by the formula given under 24(b), raffinose is probably present. Calculate sucrose and raffinose by following formulas:<sup>20</sup>

When polarizations are made at  $20^\circ$ :

$$S = \frac{0.514P - I}{0.844} \quad \text{and} \quad R = \frac{0.33P + I}{1.563}, \quad \text{in which}$$

$P$  = direct reading, normal soln;

$I$  = invert reading, normal soln;

$S$  = percentage of sucrose; and

$R$  = percentage of anhydrous raffinose.

The following formulas<sup>20</sup> are applicable at all temps. other than  $20^\circ$ .

$$S = \frac{P(0.478 + 0.0018t_2) - I(1.006 - 0.0003t_1)}{(0.908 - 0.0032t_2)(1.006 - 0.0003t_1)}, \quad \text{and}$$

$$R = \frac{P(0.43 - 0.005t_2) + I(1.006 - 0.0003t_1)}{(1.681 - 0.0059t_2)(1.006 - 0.0003t_1)}, \quad \text{in which}$$

$P$  = direct reading, normal soln;

$I$  = invert reading, normal soln;

$S$  = percentage of sucrose;

$R$  = percentage of anhydrous raffinose;

$t_1$  = temp. of the direct polarization; and

$t_2$  = temp. of invert polarization.

## 28 *SUCROSE BY DOUBLE DILUTION METHOD<sup>21</sup>—OFFICIAL*

(Substances in which the volume of the combined insoluble matter and precipitate from clarifying agents is more than 1 ml from 26 g.)

Weigh a half-normal weight of sample and dilute soln to 100 ml, using appropriate clarifier (basic Pb acetate for dark colored confectionery or molasses and alumina cream for light colored confectionery). Also weigh a normal weight of sample and dilute a second soln with the clarifier to 100 ml. Filter, and obtain direct polariscopic readings of both solns. Invert each soln as directed under 23(b) or (c) or 24(b) or (c) and obtain the respective invert readings.

The true direct polarization of sample = 4 times direct polarization of diluted soln less direct polarization of undiluted soln. The true invert polarization = 4 times invert polarization of diluted soln less invert polarization of undiluted soln. Calculate sucrose from the true polarizations thus obtained, using the formula under 23 or 24 corresponding to method of inversion used.

### SUCROSE—CHEMICAL METHODS

## 29 *From Reducing Sugars Before and After Inversion—Official*

Determine reducing sugars (clarification having been effected with neutral Pb acetate, never with basic Pb acetate) as directed under 39 and calculate to invert

sugar from **XLIII**, 9. Invert soln as directed under **23(b)** or **(c)**, or **24(b)** or **(c)**; exactly neutralize the acid; and again determine reducing sugars, but calculate them to invert sugar from the table referred to above, using invert sugar column alone. Deduct percentage of invert sugar obtained before inversion from that obtained after inversion and multiply the difference by 0.95 to obtain percentage of sucrose. Dilute the solns in both determinations so that not more than 230 mg of invert sugar is present in quantity taken for reduction. It is important that all Pb be removed from the soln with anhydrous powdered K oxalate before reduction.

#### COMMERCIAL GLUCOSE<sup>22</sup> (APPROXIMATE)—POLARIMETRIC METHODS

30

##### *Method I.—Official*

(Substances containing little or no invert sugar.)

Commercial glucose cannot be determined accurately owing to the varying quantities of dextrin, maltose, and dextrose present in the product. However, in sirups in which the quantity of invert sugar is so small as not to affect appreciably the result, commercial glucose may be estimated approximately by following formula:

$$G = \frac{(a - S)100}{211}, \text{ in which}$$

$G$  = percentage of commercial glucose solids;

$a$  = direct polarization, normal soln; and

$S$  = percentage of cane sugar.

Express results in terms of commercial glucose solids polarizing  $+211^\circ V$ . (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

31

##### *Method II.—Official*

(Substances containing invert sugar.)

Prepare an inverted half-normal soln of substance as directed under **24(b)**, except to cool the soln after inversion, make neutral to phenolphthalein with NaOH soln, slightly acidify with HCl (1+5), and treat with 5–10 ml of alumina cream before making up to mark. Filter, and polarize at  $87^\circ$  in 200 mm jacketed metal tube, preferably silver. Multiply reading by 200 and divide by factor 196 to obtain quantity of commercial glucose solids polarizing  $+211^\circ V$ . (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

#### INVERT SUGAR—CHEMICAL METHODS

##### *I. Lane-Eynon General Volumetric Method<sup>23</sup>—Tentative*

32

##### REAGENTS

*Soxhlet's modification of Fehling's soln.*—Prepare by mixing immediately before use equal volumes of (a) and (b).

(a) *Copper sulfate soln.*—Dissolve 34.639 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , dilute to 500 ml, and filter thru prepared asbestos.

(b) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salts and 50 g of NaOH in  $\text{H}_2\text{O}$ , dilute to 500 ml, allow to stand for 2 days, and filter thru prepared asbestos.

33

##### STANDARDIZATION AND METHOD OF TITRATION

Pipet accurately 10 or 25 ml of mixed Soxhlet's reagent or pipet 5 or 12.5 ml of each of Soxhlet's solns (a) and (b) into flask of 300–400 ml capacity. The quantity

of Cu taken will differ slightly between the two methods of pipetting, and the method used must be carried out consistently during standardization and determination. Prepare a standard soln of the pure sugar of such concentration that more than 15 ml and less than 50 ml will be required to reduce all the Cu. The

titer may be calculated as follows: 
$$\frac{\text{factor}}{\text{mg sugar in 1 ml}} \cdot \text{Add almost the whole of the}$$

sugar soln required to effect reduction of all the Cu, so that not more than 0.5–1 ml is required later to complete titration. Heat the cold mixture to boiling on wire gauze and maintain in moderate ebullition for 2 min., lowering flame sufficiently to avoid bumping. Without removing flame add 2–5 drops of 1% aqueous methylene blue soln and complete titration within total boiling time of ca 3 min. by small additions of sugar soln to decolorization of the indicator.

Multiply titer by number of mg in 1 ml of the standard soln to obtain the factor. Compare with the tabulated factor to determine correction, if any, to be applied to table. Small deviations from the tabulated factors may arise from variations in individual procedure or composition of reagents. If only approximate results (within 1%) are required, the standardization may be omitted, provided specifications of the analysis are rigidly observed.

34

## DETERMINATION

If approximate concentration of sugar in sample is unknown, proceed by the incremental method of titration. Add to 10 or 25 ml of Soxhlet's soln 15 ml of the sugar soln and heat to boiling over wire gauze. Boil ca 15 seconds and add rapidly further quantities of the sugar soln until only faintest perceptible blue color remains. Then add 2–5 drops of methylene blue and complete titration by adding the sugar soln dropwise. (The error resulting from this titration will not generally exceed 1%.)

For higher precision repeat titration, adding almost the whole of the sugar soln required to reduce all the Cu and proceed as directed above. In Table 15, XLIII, find factor corresponding to titer and apply correction previously determined. Estimate as follows:

$$\frac{\text{factor} \times 100}{\text{titer}} = \text{mg of sugar in 100 ml.}$$

II. Scales Method<sup>24</sup>—Tentative

(Suitable when very small quantities of sugar are present.)

35

## REAGENT

*Benedict's solns.*—Dissolve 16 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 125–150 ml of  $\text{H}_2\text{O}$ . Dissolve 150 g of Na citrate, 130 g of  $\text{Na}_2\text{CO}_3$  (anhydrous), and 10 g of  $\text{NaHCO}_3$  in ca 650 ml of  $\text{H}_2\text{O}$ , heating to accelerate soln. Combine the two solns with stirring. Cool, make to 1 liter, and filter.

36

## DETERMINATION

Transfer 20 ml of the Cu reagent to 300 ml Erlenmeyer flask fitted with 2-holed rubber stopper. Add 10 ml of sugar soln containing less than 20 mg of reducing sugar. Place over flame, bring to boiling in 4 min., and continue the boiling exactly 3 min. (Approximate conditions, flame 50 mm, cone 20 mm, asbestos gauze 30 mm above burner. If preferred, electric hot plate may be used, in which case a period of 5 min. is required to raise soln to boiling point.) At expiration of 3 min. from beginning of boiling, cool rapidly by holding under cold water faucet, and add 100 ml of acetic acid soln (24 ml of glacial acetic acid per liter) from a graduate, and

an exactly measured amount of 0.04 *N* I soln. Add 25 ml of HCl (60 ml per liter) from pipet held against side of flask, and agitate to distribute the acid rapidly. Rotate flask 1 min., or until all  $\text{Cu}_2\text{Cl}_2$  is dissolved. Titrate excess I with 0.04 *N* thiosulfate soln, using starch soln as indicator.

For amounts less than 20 mg of sugar each ml of thiosulfate will represent a constant quantity of sugar; for dextrose, ca 1.12 mg per ml. (For accurate work the analyst should determine the conversion factor for the particular conditions under which he is working by using control solns of the pure sugars under examination.)

### III. Munson and Walker General Method<sup>26</sup>—Official

37

#### REAGENTS

*Asbestos*.—Digest the asbestos, which should be the amphibole variety, with HCl (1+3) for 2–3 days. Wash free from acid, digest for similar period with 10% NaOH soln, and then treat for a few hours with hot alkaline tartrate soln (old alkaline tartrate solns that have stood for some time may be used for this purpose) of the strength used in sugar determinations. Wash asbestos free from alkali; digest for several hours with  $\text{HNO}_3$  (1+3); and, after washing free from acid, shake with  $\text{H}_2\text{O}$  into fine pulp. In preparing a Gooch crucible, make a film of asbestos  $\frac{1}{4}$ " thick and wash thoroly with  $\text{H}_2\text{O}$  to remove fine particles of asbestos. If the precipitated  $\text{Cu}_2\text{O}$  is to be weighed as such, wash crucible with 10 ml of alcohol, then with 10 ml of ether; dry for 30 min. at 100°, cool in desiccator, and weigh.

The other reagents and solns used are described under 32.

38

#### PRECIPITATION OF CUPROUS OXIDE

Transfer 25 ml of each of the  $\text{CuSO}_4$  and alkaline tartrate solns to 400 ml beaker of alkali-resistant glass and add 50 ml of the reducing sugar soln, or if a smaller volume of sugar soln is used, add  $\text{H}_2\text{O}$  to make final volume 100 ml. Heat beaker on asbestos gauze over Bunsen burner, regulate flame so that boiling begins in 4 min., and continue boiling for exactly 2 min. (It is important that these directions be strictly observed. To regulate burner for this purpose it is advisable to make preliminary tests, using 50 ml of the reagent and 50 ml of  $\text{H}_2\text{O}$  before proceeding with actual determination.) Keep beaker covered with watch-glass during heating. Filter hot soln at once thru asbestos mat in porcelain Gooch crucible, using suction. Wash precipitate of  $\text{Cu}_2\text{O}$  thoroly with  $\text{H}_2\text{O}$  at ca 60° and either weigh directly as  $\text{Cu}_2\text{O}$ , 39, or determine quantity of reduced Cu by one of methods described under 39–44, respectively. Conduct blank determination, using 50 ml of the reagent and 50 ml of  $\text{H}_2\text{O}$ , and if weight of  $\text{Cu}_2\text{O}$  obtained exceeds 0.5 mg, correct result of reducing sugar determination accordingly. The alkaline tartrate soln deteriorates on standing, and the quantity of  $\text{Cu}_2\text{O}$  obtained in the blank increases.

#### DETERMINATION OF REDUCED COPPER

39

#### *Direct Weighing of Cuprous Oxide*

(Use only for determinations in solns of reducing sugars of comparatively high purity. In products containing large quantities of mineral or organic impurities, including sucrose, determine the Cu of the  $\text{Cu}_2\text{O}$  by one of methods described under 39–44, since the  $\text{Cu}_2\text{O}$  is likely to be contaminated with foreign matter.)

Prepare a Gooch crucible as directed under 37. Collect the precipitated  $\text{Cu}_2\text{O}$  on mat as directed under 38, and wash thoroly with hot  $\text{H}_2\text{O}$ , then with 10 ml of alcohol, and finally with 10 ml of ether. Dry precipitate for 30 min. in water oven at temp. of boiling  $\text{H}_2\text{O}$ , cool, and weigh. Obtain from XLIII, 9, the weight of invert sugar equivalent to weight of  $\text{Cu}_2\text{O}$ .

The number of mg of  $\text{Cu}_2\text{O}$  reduced by a given quantity of reducing sugar varies, depending upon whether or not sucrose is present. In the tables the absence of sucrose is assumed except in the entries under invert sugar, where, in addition to the column for invert sugar alone, one column is given for mixtures of invert sugar and sucrose containing 0.4 g of total sugar in 50 ml of soln and one column for invert sugar and sucrose when the 50 ml of soln contains 2 g of total sugar. Two entries are also given under lactose and sucrose mixtures, showing proportions of 1 part lactose to 4 and 12 parts of sucrose, respectively.

*Volumetric Thiosulfate Method<sup>26</sup>—Official, first action*

40

## REAGENT

*Standard thiosulfate soln.*—Prepare a soln containing 39 g of pure  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter. Weigh accurately 0.2–0.4 g of pure Cu and transfer to 250 ml Erlenmeyer flask roughly graduated by marks at 20 ml intervals. Dissolve the Cu in 5 ml of a mixture of equal volumes of  $\text{HNO}_3$  and  $\text{H}_2\text{O}$ , dilute to 20 or 30 ml, boil to expel red fumes, add slight excess of strong Br water, and boil until Br is completely driven off. Cool, and add NaOH soln with agitation until a faint turbidity of  $\text{Cu}(\text{OH})_2$  appears (ca 7 ml of a 25% NaOH soln is required). Discharge turbidity with a few drops of acetic acid and add 2 drops in excess. Prepare a soln of 42 g of KI in 100 ml of soln made very slightly alkaline to avoid formation and oxidation of HI.

It is essential for the thiosulfate titration that the concentration of KI in the soln be carefully regulated. If soln contains less than 320 mg of Cu, at completion of titration 4.2–5 g of KI should have been added for each 100 ml of total soln. If greater quantities of Cu are present, add the KI soln slowly from buret with constant agitation in amounts proportionately greater.

Observe volume of the Cu soln and add 1 ml of KI soln for each 10 ml of the soln undergoing titration. Titrate at once with the thiosulfate soln until the brown color becomes faint. Again observe volume and add an additional volume of KI to make required concentration, noting from volume of thiosulfate the approximate Cu content of the soln. Add sufficient starch indicator, VI, 3(e), to produce a marked blue coloration. Continue titration cautiously until color changes toward end to faint lilac. As end point is approached, add the thiosulfate in fractions of drops, allowing precipitate to settle slightly after each addition. 1 ml of the thiosulfate soln = ca 10 mg of Cu.

*Alternative procedure.<sup>27</sup>*—After following all the directions given in the first paragraph, proceed as follows: Add 10 ml of the KI soln and titrate the thiosulfate to end point. Add 2 g of  $\text{NH}_4\text{SCN}$  and stir until completely dissolved. Continue titration until precipitate is perfectly white.

41

## DETERMINATION

Wash the precipitated  $\text{Cu}_2\text{O}$ , cover the Gooch crucible with watch-glass, and dissolve the oxide by means of 5 ml of  $\text{HNO}_3$  (1+1) directed under watch-glass with pipet. Collect filtrate in 250 ml Erlenmeyer flask, which is roughly graduated by marks at 20 ml intervals, and wash watch-glass and Gooch crucible free from Cu. Proceed as directed under 40, beginning, "boil to expel the red fumes . . ."

*Volumetric Permanganate Method<sup>28</sup>*

42

## REAGENTS

(a) *Potassium permanganate soln.*—Approximately 0.1573 N, and containing 4.98 g per liter. After several days' aging, filter thru asbestos or sintered glass. Standardize by one of following methods:



(1) Transfer 0.35 g of  $\text{Na}_2\text{C}_2\text{O}_4$  (dried at  $103^\circ$ ) to a 600 ml beaker. Add 250 ml of  $\text{H}_2\text{SO}_4$  (5+95) previously boiled 10 min. and cooled to  $27^\circ \pm 3^\circ$ . Stir until oxalate is dissolved. Add 29–30 ml of the  $\text{KMnO}_4$  soln at rate of 25–35 ml per minute while stirring slowly. Allow mixture to stand until pink color disappears (ca 45 seconds). Heat to  $55\text{--}60^\circ$ , and complete titration by adding permanganate until a faint pink color persists 30 seconds. Add last 0.5–1 ml dropwise, allowing each drop to become decolorized before adding the next.

Determine excess of soln (usually 0.03–0.05 ml) required to impart a pink color to same volume of acid boiled and cooled to  $55\text{--}60^\circ$ . (In potentiometric titrations correction is negligible if end point is approached slowly.)

(2) Transfer ca 0.3 g of  $\text{As}_2\text{O}_3$  (dried at  $110^\circ$ ) to a 400 ml beaker. Add 10 ml of cool soln of  $\text{NaOH}$  (20%) and allow to stand until dissolved, stirring occasionally. Add 100 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{HCl}$  (sp. gr. 1.18), and 1 drop of 0.0025  $M$   $\text{KIO}_3$ , or  $\text{KI}$ . Titrate with the  $\text{KMnO}_4$  soln until a faint pink color persists 30 seconds, adding last 1–1.5 ml dropwise and allowing each drop to become decolorized before adding the next. Determine by blank test with all reagents except  $\text{As}_2\text{O}_3$  the volume of  $\text{KMnO}_4$  (usually ca 0.03 ml) required to duplicate the pink color of end point. (The end point can also be taken with ferrous phenanthroline indicator, in which case 1 drop of a 0.025  $M$  soln of the indicator is added as the end point is approached.) Determine blank correction. The titration can also be conducted potentiometrically.

(b) *Ferric sulfate*.—Dissolve 135 g of ferric ammonium alum or 55 g of  $\text{Fe}_2(\text{SO}_4)_3$  (anhydrous) and dilute to 1 liter. Determine  $\text{Fe}_2(\text{SO}_4)_3$  in stock supply by strong ignition to  $\text{Fe}_2\text{O}_3$ . Acidify 25 ml with 10 ml of 4  $N$   $\text{H}_2\text{SO}_4$  and titrate with the  $\text{KMnO}_4$ . Add to the remaining (unacidified) soln the calculated volume of permanganate.

(c) *Ferrous phenanthroline indicator*.—Dissolve 0.7425 g of orthophenanthroline monohydrate in 25 ml of 0.025  $M$   $\text{FeSO}_4$  soln (6.95 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 1 liter).

## 43

## DETERMINATION

Filter the  $\text{Cu}_2\text{O}$  in Gooch crucible and wash beaker and precipitate thoroly. Transfer asbestos filter to beaker with aid of glass rod. Add 50 ml of the  $\text{Fe}_2(\text{SO}_4)_3$  soln and stir vigorously until  $\text{Cu}_2\text{O}$  is completely dissolved. Examine for complete solution, holding beaker above level of eye. Add 20 ml of 4  $N$   $\text{H}_2\text{SO}_4$  and titrate with standard permanganate. As end point is approached, add 1 drop of the ferrous phenanthroline indicator. At end point the brownish soln changes to green.

## 44

*Electrolytic Deposition from Nitric Acid Solution<sup>29</sup>—Tentative*

Decant the hot soln thru asbestos mat in Gooch crucible and wash beaker and precipitate thoroly with hot  $\text{H}_2\text{O}$ . Transfer asbestos mat from crucible to beaker with glass rod and rinse crucible with 14 ml of  $\text{HNO}_3$  (1+1), allowing rinsings to flow into beaker. After the  $\text{Cu}_2\text{O}$  is dissolved, dilute to 100 ml, heat to boiling, and continue boiling ca 5 min. to remove oxides of nitrogen. Cool, filter, transfer to a 250 ml beaker, and dilute to 200 ml. Add 1 drop of 0.1  $N$   $\text{HCl}$  and mix thoroly.

For electrolysis use cylindrical electrodes of Pt gauze, 1.5" and 2", respectively, in diameter and 1.75" in height, thoroly cleaned, ignited, cooled in desiccator, and weighed. Insert electrodes in  $\text{Cu}$  soln so that surface of cathode clears anode by at least 5 mm, and both electrodes almost touch the bottom of beaker. Electrolyze with current of 0.2–0.4 ampere until deposition is complete, usually overnight. Without interrupting current, slowly lower beaker and at same time wash electrodes with a stream of  $\text{H}_2\text{O}$ . Immediately immerse electrodes in another beaker of  $\text{H}_2\text{O}$ , lower beaker, and break current. Rinse cathode with ethyl alcohol and dry for a few minutes in oven at  $110^\circ$ . Cool in desiccator and weigh.

If extreme care is exercised to avoid spattering, the  $\text{Cu}_2\text{O}$  can be dissolved by allowing the  $\text{HNO}_3$  to flow down walls of crucible. Keep crucible covered as much as possible with small watch-glass. Collect filtrate in beaker and wash watch-glass and tip of pipet with jet of  $\text{H}_2\text{O}$ . Continue as directed above, beginning "dilute to 100 ml . . ."

*IV. Herzfeld Gravimetric Methods—Official*

45

*Method I.*

(Materials containing 1.5% or less of invert sugar and 98.5% or more of sucrose.)

Prepare the soln of the material to be examined to contain 20 g in 100 ml, clarify with neutral Pb acetate, and remove excess Pb with  $\text{Na}_2\text{C}_2\text{O}_4$ . Place 50 ml of the reagent, 32, and 50 ml of the sugar soln in 250 ml beaker. Heat this mixture at such a rate that ca 4 min. is required to bring it to boiling point and boil for exactly 2 min. Add 100 ml of cold recently boiled  $\text{H}_2\text{O}$ . Filter immediately thru asbestos, 37, and determine Cu by one of methods described under 39–44. Obtain corresponding percentage of invert sugar from XLIII, 10.

46

*Method II.*

(For materials containing more than 1.5% of invert sugar and less than 98.5% of sucrose.)

Prepare a soln of suitable concentration of material to be examined, clarify with neutral Pb acetate, and remove excess of Pb. Prepare series of solns in large test tubes by adding 1, 2, 3, 4, and 5 ml of this soln to each tube successively. Add 5 ml of the reagent, 32, to each, heat to boiling, boil 2 min., and filter. Note the volume of sugar soln that gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar soln in 100 ml flask, dilute to mark, and mix well. Use 50 ml of the soln for the determination, and proceed as directed under 45. For calculation of result use following formulas and the table of factors of Meissl and Hiller, XLIII, 11.

Let  $Cu$  = weight of Cu obtained;

$P$  = polarization of sample;

$W$  = weight of sample in the 50 ml of the soln used for determination; and

$F$  = factor obtained from table for conversion of Cu to invert sugar.

Then  $\frac{Cu}{2} = Z$ , approximate weight of invert sugar;

$Z \times \frac{100}{W} = Y$ , approximate percentage of invert sugar;

$\frac{100 P}{P + Y} = R$ , approximate percentage of sucrose in mixture of sugars;

$100 - R = I$ , approximate percentage of invert sugar; and

$\frac{Cu F}{W}$  = percentage of invert sugar.

Use the factor  $F$  for calculating Cu to invert sugar, Table 11, XLIII. *Example:* The polarization of a sugar is 86.4, and 50 ml of soln containing 3.256 g of sample gives 0.290 g of Cu.

$$\frac{Cu}{2} = \frac{0.290}{2} = 0.145 = Z.$$

$$\frac{Z \times 100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = Y.$$

$$\frac{100 P}{P + Y} = \frac{8460}{86.4 + 4.45} = 9.51 = R.$$

$$100 - R = 100 - 95.1 = I = 4.9.$$

$$R:I = 95.1:4.9.$$

By consulting the table it will be seen that the vertical column headed 150 is nearest to *Z*, 145, and the horizontal column headed 95:5 is nearest to the ratio of *R* to *I*, 95.1:4.9. Where these columns meet is found the factor 51.2, which enters into the final calculation:

$$\frac{CuF}{W} = \frac{0.290 \times 51.2}{3.256} = 4.56\% \text{ of invert sugar.}$$

#### DEXTROSE—CHEMICAL METHODS

##### 47 *Lane-Eynon General Volumetric Method—Tentative*

Proceed as directed under 34, referring titer to **XLIII**, 15 or 16.

##### 48 *Munson-Walker General Gravimetric Method—Official*

Proceed as directed under 38 and obtain from **XLIII**, 9, the weight of dextrose equivalent to weight of Cu reduced.

##### *Allihn Gravimetric Method—Official*

49

#### REAGENTS

(a) *Copper sulfate soln.*—Dissolve 34.639 g of  $CuSO_4 \cdot 5H_2O$  in  $H_2O$  and dilute to 500 ml.

(b) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salts and 125 g of KOH in  $H_2O$  and dilute to 500 ml.

50

#### DETERMINATION

Place 30 ml of the  $CuSO_4$  soln, 30 ml of the alkaline tartrate soln, and 60 ml of  $H_2O$  in beaker, and heat to boiling. Add 25 ml of the soln of the material to be examined containing not more than 0.25 g of dextrose, and boil exactly 2 min., keeping beaker covered. Filter immediately thru asbestos and obtain weight of Cu by one of methods given under 39-44, respectively. Obtain corresponding weight of dextrose from **XLIII**, 14.

#### LEVULOSE—CHEMICAL METHODS

51

##### *Lane-Eynon General Volumetric Method*

Proceed as directed under 34, referring titer to **XLIII**, 15 or 16.

##### *Jackson-Mathews Modification of Nyns Selective Method<sup>30</sup>—Tentative*

52

#### REAGENT

*Ost's soln.*—Dissolve 250 g of  $K_2CO_3$  (anhydrous) in ca 700 ml of hot  $H_2O$ , add 100 g of pulverized  $KHCO_3$ , and agitate mixture until completely dissolved. Cool, and add with very vigorous agitation a soln of 25.3 g of  $CuSO_4 \cdot 5H_2O$  in 100-150 ml of  $H_2O$ . Make to 1 liter and filter.

Transfer 50 ml of Ost's soln to 150 ml Erlenmeyer flask and add by means of accurately graduated pipet a volume of the soln to be analyzed that contains not more than 92 mg of levulose or its equivalent of a levulose-dextrose mixture, remembering that dextrose has about one-twelfth the reducing power of levulose. Add enough  $H_2O$  to make total volume 70 ml. Immerse in water bath, regulated preferably within  $0.1^\circ$ , at  $55^\circ$ . Digest for exactly 75 min., agitating with rotary motion at intervals of 10 or 15 min.

Filter the precipitated  $Cu_2O$  on a closely packed Gooch crucible and wash flask and precipitate thoroly without attempting to transfer the precipitate quantitatively. Determine Cu by one of methods described under 39-44. As it is usually difficult to transfer the Cu precipitate quantitatively from the Erlenmeyer flask, select a method of Cu analysis in which the total Cu is dissolved in  $HNO_3$  and determined by electrolysis or thiosulfate titration, or in a  $Fe_2(SO_4)_3$  soln followed by permanganate titration as directed in 43.

See Table 13, **XLIII**, for the levulose equivalent. If sample contained glucose in addition to levulose the analytical result is not true but "apparent" levulose, as glucose has an appreciable reducing action under the conditions of the analysis. To determine the correction for glucose analyze the sample also for total reducing sugar and compute the true glucose and levulose by a series of approximations. Calculate percentage of reducing sugar in original sample and similarly percentage of "apparent" levulose. The difference between these two percentages is the "apparent" glucose. Divide the apparent glucose by the factor 12.4 and deduct result from apparent levulose to obtain a new approximation to the true levulose. Deduct the new levulose percentage from the total reducing sugar percentage to obtain a more correct value for true glucose and again divide by 12.4. Deduct quotient from the original value of the "apparent" levulose and continue the approximation in the same manner until the percentage of levulose remains essentially unaltered by two successive approximations.

If the original sample contained sucrose, determine it by means of the Clerget procedure, 24. Correct the Cu for the reducing action of sucrose before referring to the table, **XLIII**, 13. 1, 2, 3, 4, and 5 g of sucrose precipitate under the conditions of the analysis 3.3, 5.7, 7.4, 8.5, 9.0 mg of Cu, respectively.

#### MALTOSE—CHEMICAL METHODS

##### 54 *Lane-Eynon General Volumetric Method—Tentative*

Proceed as directed under 34, referring titer to **XLIII**, 15 or 16.

##### 55 *Munson-Walker General Gravimetric Method—Official*

Proceed as directed under 38 and obtain from **XLIII**, 9, the weight of maltose equivalent to weight of Cu reduced.

#### LACTOSE—CHEMICAL METHODS

##### 56 *Lane-Eynon General Volumetric Method—Tentative*

Proceed as directed under 34, referring titer to **XLIII**, 15 or 16.

##### 57 *Munson-Walker General Gravimetric Method—Official*

Proceed as directed under 38 and obtain from **XLIII**, 9, the weight of lactose equivalent to weight of Cu reduced.

58

## REDUCING SUGARS OTHER THAN DEXTROSE—OFFICIAL

Proceed as directed under 50. Multiply weight of dextrose found by following factors: arabinose, 0.969; xylose, 1.017; and galactose, 1.114.

MICROMETHOD FOR REDUCING SUGARS<sup>1</sup>—TENTATIVE

59

## REAGENT

Dissolve 12 g of Rochelle salt, 20 g of  $\text{Na}_2\text{CO}_3$ , and 25 g of  $\text{NaHCO}_3$  in ca 500 ml of  $\text{H}_2\text{O}$  and into this soln pour with stirring 6.5 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  dissolved in ca 100 ml of  $\text{H}_2\text{O}$ ; add a soln composed of 10 g of KI, 0.80 g of  $\text{KIO}_3$ , and 18 g of  $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ , and dilute to 1 liter. Only the  $\text{KIO}_3$  need be weighed accurately.

60

## DETERMINATION

Measure 5 ml (accurately) of the reagent into test tube (25×250 mm) and add 5 ml of the sugar soln containing not less than 0.1 mg nor more than 2 mg of dextrose. Mix by gentle shaking, cover tube, and keep in boiling water bath 15 min. Cool in a water bath to 35–40°. Add with agitation 1 ml of 5 *N*  $\text{H}_2\text{SO}_4$  and see that all  $\text{Cu}_2\text{O}$  is dissolved. After ca 2 min. titrate with 0.005 *N* thiosulfate soln, using starch indicator. Determine blank titration on 5 ml of reagent after heating with 5 ml of  $\text{H}_2\text{O}$ . Deduct the titer of a determination from that of the blank and refer result to the table, 61.

NOTE: It is important to run control analyses with pure dextrose and apply a correction for any deviation from the tabulated equivalents.

61

*Somogyi's<sup>1</sup> Dextrose-Thiosulfate Equivalents*

(Quantities of dextrose corresponding to titration values when 5 ml soln and 5 ml of copper reagent are heated in a water bath for 15 minutes.)

ML OF 0.005 <i>N</i> THIO- SULFATE	TENTHS OF 1 ML OF 0.005 <i>N</i> SODIUM THIOSULFATE									
	0	1	2	3	4	5	6	7	8	9
	DEXTROSE IN 5 ML SOLUTION (MG)									
0			.11	.12	.13	.15	.16	.17	.18	.20
1	.21	.22	.23	.25	.26	.27	.28	.29	.31	.32
2	.33	.34	.35	.36	.38	.39	.40	.41	.42	.43
3	.45	.46	.47	.485	.495	.505	.515	.530	.540	.550
4	.565	.575	.585	.595	.605	.620	.630	.640	.650	.660
5	.670	.685	.695	.705	.715	.730	.740	.750	.760	.770
6	.785	.795	.805	.815	.825	.840	.850	.860	.870	.880
7	.895	.905	.915	.925	.935	.950	.960	.970	.980	.995
8	1.005	1.015	1.025	1.035	1.050	1.060	1.070	1.080	1.090	1.105
9	1.115	1.125	1.135	1.115	1.116	1.117	1.185	1.195	1.205	1.215
10	1.225	1.24	1.25	1.26	1.27	1.28	1.295	1.305	1.315	1.325
11	1.335	1.35	1.36	1.37	1.38	1.395	1.405	1.415	1.425	1.44
12	1.450	1.460	1.470	1.480	1.495	1.505	1.515	1.525	1.54	1.55
13	1.560	1.570	1.580	1.590	1.605	1.615	1.63	1.640	1.650	1.660
14	1.670	1.685	1.695	1.705	1.715	1.725	1.735	1.750	1.760	1.170
15	1.780	1.795	1.805	1.815	1.825	1.835	1.850	1.860	1.870	1.880
16	1.89	1.905	1.915	1.930	1.940	1.950	1.960	1.970	1.98	1.990
17	2.00									

<sup>1</sup> M. Somogyi, *J. Biol. Chem.*, 70, 607 (1926). 1 ml of 0.005 *N* thiosulfate = 0.318 mg. of Cu.

## CONFECTIONERY

62

## PREPARATION OF SAMPLE—OFFICIAL

If the composition of the entire sample is desired, grind and mix thoroly. If the sample is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with a knife or other mechanical means as completely as possible and grind and mix each portion thoroly.

63

## MOISTURE—OFFICIAL.—See 2, 3, 4, or 5.

64

## ASH—OFFICIAL.—See 9 or 10.

65

## MINERAL CONSTITUENTS—OFFICIAL.—See XII.

66

## SOLUBLE AND INSOLUBLE ASH—OFFICIAL.—See 13.

67

## ALKALINITY OF SOLUBLE ASH—OFFICIAL.—See 14.

68

## ALKALINITY OF INSOLUBLE ASH—OFFICIAL.—See 15.

69

## MINERAL ADULTERANTS IN THE ASH—TENTATIVE.—See 16.

70

## NITROGEN—OFFICIAL

Determine N in 2–5 g of the material as directed under II, 21, 22, or 23, using a larger quantity of  $\text{H}_2\text{SO}_4$  if necessary for complete digestion.

## SUCROSE—POLARIMETRIC METHODS

71

*In Absence of Raffinose—Official.—See 23, 24, or 28.*

## SUCROSE—CHEMICAL METHODS

72

*By Reducing Sugars Before and After Inversion—Official.—See 29.*

73

## COMMERCIAL GLUCOSE—OFFICIAL.—See 30 or 31.

74

## STARCH—TENTATIVE

Measure 25 ml of a soln of uniform mixture (representing 5 g of sample) into 300 ml beaker, or introduce into the beaker 5 g of the finely ground sample (previously extracted with ether if sample contains much fat); add sufficient  $\text{H}_2\text{O}$  to make volume 100 ml; heat to ca  $60^\circ$  (avoiding if possible gelatinizing the starch); and allow to stand ca 1 hour, stirring frequently to secure complete soln of the sugars. Transfer to wide-mouthed bottle, rinse beaker with a little warm  $\text{H}_2\text{O}$ , and cool. Add an equal volume of 95% alcohol, mix, and allow to stand at least an hour. Centrifuge until precipitate is closely packed on bottom of bottle and decant supernatant liquid thru hardened filter. Wash precipitate with successive 50 ml portions of alcohol, 50% by volume, by centrifuging and decanting thru filter until 3 or 4 drops of washings give no test for sugar with alpha-naphthol as described under 125. Transfer residue from bottle and hardened filter to a large flask and determine starch as directed under XXVII, 32.

## ETHER EXTRACT

75

*I. Continuous Extraction Method—Tentative*

(1) Measure 25 ml of a 20% mixture or soln into a very thin, readily frangible glass evaporating shell, or a thin lead or tin foil dish containing 5–7 g of freshly ignited asbestos fiber; or (2) if possible to obtain a uniform sample, weigh 5 g of the mixed finely divided sample into dish and wash with  $\text{H}_2\text{O}$  upon the asbestos in the evaporating shell using, if necessary, a small portion of the asbestos fiber on a stir-

ring rod to transfer last traces of sample from dish to shell. Dry to constant weight at 100°, cool, wrap glass dish loosely in smooth paper, crush into rather small fragments between the fingers, and carefully transfer crushed mass, including paper, to extraction tube or fat extraction cartridge. If metal dish is used, cut it into small pieces and place in extraction tube. Extract with anhydrous ether or petroleum benzin (b.p. 45–60° and without weighable residue) in continuous extraction apparatus for at least 25 hours. In most cases it is advisable to remove the substance from the extractor after the first 12 hours, grind with sand to fine powder, and re-extract for remaining 13 hours. Transfer extract to weighed flask, evaporate solvent, and dry to constant weight at 100°.

## 76

*II. Roese-Gottlieb Method—Tentative*

Introduce 4 g of the material, or a quantity of a uniform soln equivalent to this weight of the dry substance, into a Röhrig tube or a similar apparatus; make up to volume of 10 ml with H<sub>2</sub>O, add 1.25 ml of NH<sub>4</sub>OH, and mix thoroly. Add 10 ml of alcohol and mix; then add 25 ml of ether and shake vigorously for half a minute; and finally add 25 ml of petroleum benzin (b.p. below 60°) and shake again for half a minute. Allow to stand for 20 min. or until separation of liquids is complete. Draw off as much as possible of the benzin-fat soln (usually 0.5–0.8 ml will be left) into weighed flask thru small, rapid filter. Weigh flask with a similar one as a counterpoise. Again extract liquid remaining in tube, this time with 15 ml each of ether and petroleum benzin; shake vigorously half a minute with each solvent and allow to settle. Proceed as above, washing tip of spigot and filter with a few ml of a mixture of equal parts of the two solvents (previously mixed and freed from deposited H<sub>2</sub>O). For a greater degree of accuracy the extraction must be repeated. If the previous solvent-fat solns have been drawn off closely, this third extraction usually yields not more than ca 1 mg of fat, or ca 0.02% on a 4 g charge. Evaporate the solvent slowly on steam bath and then dry fat in boiling water oven until loss in weight ceases. Test purity of the fat by dissolving in a little petroleum benzin. Should a residue remain, wash fat out completely with petroleum benzin, dry residue, weight, and deduct weight.

## 77

## PARAFFIN—TENTATIVE

Add to the solvent extract in flask, 75 or 76, 10 ml of alcohol and 2 ml of NaOH soln (1+1); connect flask with reflux condenser; and heat for an hour on water bath, or until saponification is complete. Remove condenser and allow flask to remain on bath until the alcohol is evaporated and the residue is dry. Dissolve residue as completely as possible in ca 40 ml of H<sub>2</sub>O and heat on bath, shaking frequently. Wash into separatory funnel, cool, and extract with 4 successive portions of petroleum benzin, collecting extracts in weighed flask or capsule. Evaporate the petroleum benzin and dry to constant weight at 100°. Any phytosterol or cholesterol present in the fat would be extracted with the paraffin, but the quantity is so insignificant that it may generally be disregarded.

## 78 ALCOHOL IN SIRUPS USED IN CONFECTIONERY ("BRANDY DROPS")—OFFICIAL

Collect in a beaker the sirup from a sufficient number of pieces to yield 30–50 g, strain into weighed beaker, and weigh. Introduce the sirup into 250–300 ml distillation flask, dilute with half its volume of H<sub>2</sub>O, attach flask to a vertical condenser, and distil almost 50 ml, or as much of liquid as possible without causing charring. Foaming may be prevented by adding to contents of distillation flask a little tannin, or a piece of paraffin about the size of a pea. Cool distillate, make up to volume with

H<sub>2</sub>O, and mix well. Determine sp. gr. as directed under XIV, 3. Calculate percentage of alcohol by weight in candy filling, using Table 19, XLIII.

79

COLORING MATTERS—TENTATIVE.—See XXI.

80

METALS—TENTATIVE.—See XXIX.

HONEY<sup>32</sup>

81

## PREPARATION OF SAMPLE—OFFICIAL

(a) *Liquid or strained honey*.—If the sample is free from granulation, mix thoroly by stirring or shaking before weighing portions for the analytical determination. If the honey is granulated, place container, having stopper loose, in water bath and heat at temp. not exceeding 50° with occasional stirring until the sugar crystals dissolve. Mix thoroly, cool, and weigh portions for the analytical determinations. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat sample to 40° in water bath and strain thru cheese-cloth in hot water funnel before weighing portions for analysis.

(b) *Comb honey*.—Cut across the top of comb, if sealed, and separate completely from the comb by straining thru 40-mesh sieve. When portions of comb or wax pass thru sieve, heat sample as directed in (a) and strain thru cloth. If the honey is granulated in the comb, heat until wax is liquefied; stir, cool, and remove wax.

82

## MOISTURE—OFFICIAL

Proceed as directed under 4 or 5, using a weighed quantity of sample sufficient to yield ca 1 g of solids; adding, if necessary, a few ml of H<sub>2</sub>O to incorporate thoroly with the sand; and drying at 70° under pressure of not to exceed 50 mm of Hg.

83

## ASH—OFFICIAL

Weigh 5–10 g of honey into Pt dish, add a few drops of pure olive oil to prevent spattering, heat carefully until swelling ceases, and ignite at temp. not above dull redness (ca 600°) until a white ash is obtained.

84

## SOLUBLE ASH—OFFICIAL.—See 13.

85

## ALKALINITY OF SOLUBLE ASH—OFFICIAL.—See 14.

86

## DIRECT POLARIZATION—TENTATIVE

(a) *Immediate direct polarization*.—Transfer 26 g of the honey to a 100 ml flask with H<sub>2</sub>O, add 5 ml of alumina cream, dilute to mark with H<sub>2</sub>O at 20°, filter, and polarize immediately in 200 mm tube.

(b) *Constant direct polarization*.—Complete mutarotation as directed under 21. If necessary to conserve sample, the soln from tube used in immediate direct polarization (a) may be returned to flask. Make final reading at 20° in 200 mm tube.

(c) *Mutarotation*.—The difference between (a) and (b) is a measure of the mutarotation.

(d) *Direct polarization at 87°*.—Polarize the soln obtained under (b) at 87° in a jacketed 200 mm metal tube, preferably silver.

87

## INVERT POLARIZATION—TENTATIVE

(a) *At 20°*.—Invert 50 ml of soln, 86, as directed under 23(b) or (c) or 24(b) or (c), and polarize at 20° in 200 mm tube.

(b) *At 87°*.—Polarize soln, (a), at 87° in 200 mm metal tube, preferably silver.



88

## REDUCING SUGARS—OFFICIAL

Dilute 10 ml of soln, 86, to 250 ml and determine reducing sugars in 25 ml of this soln by one of methods given, 34 or 38. Calculate result to percentage of invert sugar.

89

## SUCROSE—OFFICIAL

(a) Calculate from the data given in 86(b) and 87(a) if inversion is conducted as directed under 23(b) or (c). Use the formula given in 23(b).

(b) Proceed as directed under 29. Determine reducing sugars after inversion by diluting 10 ml of soln, 87, with small quantity of  $H_2O$ , neutralizing with  $Na_2CO_3$ , and making up to 250 ml with  $H_2O$ . Use 50 ml of this soln, making determination as directed in 88.

90

## LEVULOSE—TENTATIVE

Multiply direct reading at  $87^\circ$ , 86(d), by 1.0315 and subtract product from constant direct polarization at  $20^\circ$ , 86(b); divide difference by 2.3919 to obtain grams of levulose in normal weight of the honey. From this figure calculate percentage of levulose in original sample. Or determine levulose selectively by 52.

91

## DEXTROSE—TENTATIVE

To obtain the approximate percentage of dextrose, subtract percentage of levulose, 90, from percentage of invert sugar, 88.

The dextrose can be determined more accurately by multiplying percentage of levulose, 90, by factor 0.915, which gives its dextrose equivalent in Cu reducing power. Subtract figure obtained from that of reducing sugars, 88, calculated as dextrose, to obtain percentage of dextrose in sample. Owing to difference in reducing powers of different sugars, the sum of the dextrose thus found and the levulose, 90, will be greater than the quantity of invert sugar obtained under 88.

92

## DEXTRIN (APPROXIMATE)—TENTATIVE

Using not more than 4 ml of  $H_2O$ , transfer 8 g of sample (4 g in the case of dark colored honeydew honey) to a 100 ml flask by allowing sample to drain from weighing dish into flask and then dissolving residue in 2 ml of  $H_2O$ . After adding this soln to contents of flask, rinse weighing dish with two 1 ml portions of  $H_2O$ , adding a few ml of absolute alcohol each time before decanting. Fill flask to mark with absolute alcohol, shaking constantly. Set flask aside until the dextrin has collected on sides and bottom and liquid is clear. Decant the clear liquid thru filter paper and wash residue in the flask with 10 ml of alcohol, pouring washings thru same filter. Dissolve the dextrin in the flask with boiling  $H_2O$  and filter thru the filter paper already used, receiving filtrate in weighed dish prepared as directed under 5. Rinse flask and wash filter a number of times with small portions of hot  $H_2O$ , evaporate on water bath, and dry to constant weight at  $70^\circ$  under a pressure of not to exceed 50 mm of Hg.

After determining weight of alcohol precipitate, dissolve latter in  $H_2O$  and make up to definite volume, using 50 ml of  $H_2O$  for each 0.5 g of precipitate or part thereof.

Determine reducing sugars in the soln both before and after inversion as directed under 29, expressing results as invert sugar. Calculate sucrose from results thus obtained and subtract sum of the reducing sugars before inversion and sucrose from weight of the total alcohol precipitate to obtain weight of dextrin.

93

## FREE ACID—OFFICIAL

Dissolve 10 g of the honey in  $H_2O$  and titrate with 0.1 N NaOH soln, using

phenolphthalein indicator. Express results in terms of ml of 0.1 N NaOH required to neutralize 100 g of sample.

#### COMMERCIAL GLUCOSE

94

##### *Qualitative Test—Tentative*

Dilute the honey with  $H_2O$  in proportion of 1 to 1 and add a few ml of I soln (1 g of I, 3 g of KI, 50 ml of  $H_2O$ ). In the presence of commercial glucose the soln turns red or violet, the depth and character of the color depending upon the quality and nature of the glucose used. A blank test with a pure honey of about the same color should be made in order to secure an accurate color comparison. Should the honey be dark and the percentage of glucose very small, precipitate the dextrin that may be present by adding several volumes of alcohol. Allow to stand until the precipitate settles (do not filter), decant liquid, dissolve residue of dextrans in hot  $H_2O$ , cool, and apply the above test to this soln. A negative result is not proof of the absence of commercial glucose, as some glucose, especially of high conversion, does not give any reaction with I.<sup>33</sup>

95

##### *Quantitative Method—Tentative*

An approximate determination can be made by Browne's formula as follows: Multiply difference in polarizations of invert soln at 20° and 87°, 87, by 77 and divide this product by percentage of invert sugar found in sample after inversion. Multiply quotient by 100 and divide product by 26.7 to obtain percentage of honey in sample; 100% minus percentage of honey gives percentage of glucose.<sup>33</sup>

#### COMMERCIAL INVERT SUGAR<sup>34</sup>

##### *Resorcinol Test<sup>35</sup>—Tentative*

96

##### REAGENT

*Resorcinol soln.*—Dissolve 1 g of resublimed resorcinol in 100 ml of HCl (sp. gr. 1.18–1.19).

97

##### DETERMINATION

Introduce 10 ml of a 50% honey soln into a test tube and add 5 ml of ether. Shake gently and allow to stand until ether layer is clear. Transfer 2 ml of this clear ether soln to small test tube and add a large drop of the recently prepared resorcinol soln. Shake, and note color. A cherry red color appearing within a minute indicates presence of commercial invert sugar. Yellow to salmon shades have no significance.

##### *Aniline Chloride Test<sup>36</sup>—Tentative*

98

##### REAGENT

*Aniline chloride soln.*—To 100 ml of C. P. aniline add 30 ml of 25% HCl.

99

##### DETERMINATION

Introduce 5 g of the honey into a porcelain dish and add while stirring 2.5 ml of the recently prepared aniline reagent. In the presence of commercial invert sugar, the reagent assumes within a minute an orange-red color turning dark red. Yellow to salmon shades have no significance.

The resorcinol test and the aniline chloride test, when negative, may not be regarded as conclusive evidence of the absence of commercial invert sugar sirup in honey.

## 100

DIASTASE<sup>37</sup>—TENTATIVE

Mix 1 part of honey with 2 parts of sterile H<sub>2</sub>O. Treat 10 ml of this soln with 1 ml of 1% soluble starch soln and digest at 45° for an hour. Test mixture with 1 ml of I soln (1 g of I, 2 g of KI, 300 ml of H<sub>2</sub>O). Treat another 10 ml portion of the honey soln, mixed with 1 ml of the soluble starch soln without heating to 45°, with the reagent and compare colors produced. If the original honey has not been heated sufficiently to destroy the diastase, an olive-green or brown coloration will be produced in the mixture that has been heated at 45°. Heated or artificial honey becomes blue.

MAPLE PRODUCTS<sup>38</sup>

## 101

## PREPARATION OF SAMPLE

(a) *Maple Sirup—Official*

1. *For solids determination.*—If the sample contains no sugar crystals or suspended matter, decant sufficient of the clear sirup for use in the determination. If sugar crystals are present, redissolve them by heating. If suspended matter is present, filter the sample thru cotton wool.

2. *For other determinations.*—If sugar crystals are present, redissolve them by heating. If other sediment is present, distribute it evenly thru the sirup by shaking. Transfer ca 100 ml of the sirup, with its suspended sediment, to a casserole or beaker, add  $\frac{1}{4}$  the volume of H<sub>2</sub>O, and evaporate over a flame. When the temp. of the boiling sirup approaches 104°, draw a small quantity into a thin-walled pipet of ca 1 ml capacity, and cool to room temp. in running H<sub>2</sub>O. Wipe outside of pipet, allow the possibly diluted sirup in the point to escape, and make a refractometric measurement of the solids content of the cooled sirup. Repeat this procedure from time to time until a reading is obtained corresponding to 64.5% solids ( $n_{20} = 1.4521$ ), or to such other value as in the experience of the analyst will give a filtered sirup of 65.0% solids. Filter sirup thru a filter that will allow the 100 ml to pass within 5 min. and adjust filtrate to 65.0  $\pm$  0.5% solids (refractometric) by thoro mixing with the appropriate quantity of H<sub>2</sub>O.

(b) *Maple Sugar and Other Solid or Semi-Solid Products—Tentative*

1. *For moisture and solids determination.*—Grind in a mortar, if necessary, and mix thoroly.

2. *For other determinations.*—Prepare a sirup by dissolving ca 100 g of sample in 150 ml of hot H<sub>2</sub>O, boil until temp. approaches 104° and complete preparation of resulting sirup as directed in (a) 2, commencing "draw a small quantity into a thin-walled pipet."

## MOISTURE OR SOLIDS—OFFICIAL

## 102

*Maple Sugar*

Proceed as directed under 2, or preferably 3, using sample prepared as directed under 1.

## 103

*Maple Sirup, Maple Cream, etc.*

Proceed as directed under 3, 4, or 8, using sample prepared as directed under 101.

## 104

## ASH—OFFICIAL

Using 5 g of the prepared sirup, 101(a) (2) and 101(b) (2), proceed as directed under 9 or 10.

- 105 SOLUBLE AND INSOLUBLE ASH—OFFICIAL.—*See* 13.  
106 ALKALINITY OF SOLUBLE ASH—OFFICIAL.—*See* 14.  
107 ALKALINITY OF INSOLUBLE ASH—OFFICIAL.—*See* 15.  
108 ALKALINITY OF TOTAL ASH—OFFICIAL

Add the alkalinities of the soluble and insoluble portions (106 and 107).

- 109 METALS—TENTATIVE.—*See* XXIX.

POLARIZATION—OFFICIAL

- 110 *Direct Polarization.*—*See* 23(a) or 24(a)

- 111 *Invert Polarization*

(a) *At 20°.*—Proceed as directed under 23(b) or (c) or 24(b) or (c).

(b) *At 87°.*—Proceed as directed under 31.

- 112 SUCROSE—POLARIMETRIC METHODS—OFFICIAL

Proceed as directed under 23 or 24, or calculate from the results of 110 and 111, using the appropriate formula from 23 or 24.

SUCROSE—CHEMICAL METHODS

- 113 *By Reducing Sugars Before and After Inversion.*—*Official.*—*See* 29.

- 114 REDUCING SUGARS AS INVERT SUGAR—OFFICIAL

(a) *Before inversion.*—Proceed as directed under 34 or 38, using an aliquot of the soln used for direct polarization, 110, and only neutral Pb acetate for clarification.

(b) *After inversion.*—Proceed as directed under 34 or 38, using an aliquot of the soln used for the invert polarization, 111(a), and only neutral Pb acetate for clarification.

- 115 COMMERCIAL GLUCOSE—OFFICIAL.—*See* 30 or 31.

LEAD NUMBER

*Canadian Lead Number*<sup>39</sup> (*Fowler Modification*)—*Official*

- 116 REAGENT

*Standard basic lead acetate soln.*—Activate litharge by heating it to 650–670° for 2.5–3 hours in a muffle. (The cooled product should be lemon color.) In a 500 ml Erlenmeyer flask provided with a return condenser boil 80 g of normal Pb acetate crystals and 40 g of the freshly activated litharge with 250 g of H<sub>2</sub>O for 45 min. Cool, filter off any residue, and dilute with recently boiled H<sub>2</sub>O to a density of 1.25 at 20°.

- 117 DETERMINATION

Weigh the quantity of sirup containing 25 g of dry matter, transfer to a 100 ml flask, and make up to mark at 20°, or use the soln in which the conductivity value has been determined, 121. Pipet 20 ml into large test tube, add 2 ml of the standard basic Pb acetate soln, cork, and allow to stand 2 hours.

Filter with suction on a 25 ml tared Gooch, having an asbestos mat at least 3 mm thick. When nearly all liquid has run thru, fill crucible with cold H<sub>2</sub>O. Repeat to a total of 4 washings, taking care to prevent formation of fissures in the precipitate by keeping it covered with H<sub>2</sub>O and avoiding too great suction. Dry at 100°, weigh, and multiply weight by 20.

NOTE: Filtration may be facilitated and the necessity of keeping the precipitate in the crucible covered with  $\text{H}_2\text{O}$  obviated by stirring a weighed quantity (0.5 g or less) of dry asbestos fiber with the precipitate and supernatant liquid shortly before filtration.

*Winton Lead Number*<sup>40</sup>—Official

118

## REAGENT

*Standard basic lead acetate soln.*—To a measured volume of the reagent prepared for determination of the Canadian lead number, 116, add 4 volumes of  $\text{H}_2\text{O}$ , and filter. Run a blank with each set of determinations.

119

## DETERMINATION OF LEAD IN THE BLANK

Transfer 25 ml of the standard basic Pb acetate to a 100 ml flask, add a few drops of glacial acetic acid, and make up to mark with  $\text{H}_2\text{O}$ . Shake, and determine  $\text{PbSO}_4$  in 10 ml of the soln as directed under 120. The use of acetic acid is imperative in order to retain all Pb in soln when the reagent is diluted with  $\text{H}_2\text{O}$ .

120

## DETERMINATION

Transfer 25 g of the sample to a 100 ml flask by means of  $\text{H}_2\text{O}$ . Add 25 ml of the standard basic Pb acetate soln and shake. Fill to mark, shake, and allow to stand for at least 3 hours before filtering. Pipet 10 ml of the clear filtrate into 250 ml beaker, add 40 ml of  $\text{H}_2\text{O}$  and 1 ml of  $\text{H}_2\text{SO}_4$ , shake, and add 100 ml of alcohol. Allow to stand overnight, filter on weighed Gooch crucible, wash with alcohol, dry in water oven, and ignite in muffle or over Bunsen burner, applying heat gradually at first and avoiding reducing flame. Cool, and weigh. Subtract the weight of  $\text{PbSO}_4$  so found from the weight of  $\text{PbSO}_4$  found in the blank, 119, and multiply by factor 27.33. The use of this factor gives the Pb number directly (without various calculations otherwise required).

CONDUCTIVITY VALUE<sup>41</sup>—OFFICIAL

121

## APPARATUS

(1) *Conductivity cell.*—Should be made of resistance glass with platinized Pt electrodes firmly fixed and adequately protected from displacement. These electrodes may be sealed into a vessel into which the soln under examination may be run and subsequently drawn off (*Zerban type*), or attached to a support so that they can be lowered into a cylinder (or 100 ml beaker) containing the soln (dipping type). Provide the cell with a thermometer graduated in tenths of degrees and covering 20–30°, and place the bulb in the immediate vicinity of the electrodes. The cell constant should be ca 0.15.

(2) *Galvanometer or a microphone hummer (or an induction coil) and a sensitive telephone receiver.*

(3) *Suitable source of current.*—Dry or storage cells if a hummer or induction coil is used; 110 volt alternating current if a galvanometer is used.

(4) *Resistances of 10 and 100 ohms.*—Should be fixed and accurate.

(5) *Slide wire or Wheatstone bridge.*

(6) *Device for control of the temp. of the cell to within  $\pm 0.1^\circ$ .*—This may consist of a thermostat or of a vessel into which  $\text{H}_2\text{O}$  of suitable temp. may be run so as to adjust the cell contents to 25°.

122

## DETERMINATION OF THE CELL CONSTANT

Prepare solns of 0.3728 and 0.7456 g of dry KCl in  $\text{H}_2\text{O}$ , which offers a resistance

of at least 25,000 ohms in the cell, and make to mark at 20–25° in 500 ml volumetric flasks. Fill cell with the more dilute (0.01 *M*) soln, adjust to 25°±0.1°, measure the electrical resistance, and multiply the number of ohms by 141.2. Rinse with the stronger (0.02 *M*) soln, fill cell with this soln, measure its resistance at 25°, and multiply by 276.1. Average the two results.

123

## DETERMINATION

Weigh out a quantity of sirup that contains 25 g of dry matter, transfer to a 100 ml volumetric flask with warm H<sub>2</sub>O of the same quality as that used in the determination of the cell constant, cool to 25°, make to mark, and measure the resistance in the cell at 25°±0.1°. Divide the cell constant by the number of ohms found.

124

MALIC ACID VALUE (COWLES)<sup>42</sup>—TENTATIVE

Weigh 6.7 g of the sample into a 200 ml beaker; add 5 ml of H<sub>2</sub>O, then 2 ml of a 10% Ca acetate soln; and stir. Add, gradually and with constant stirring, 100 ml of 95% alcohol and agitate the soln until the precipitate settles, or let stand until the supernatant liquid is clear. Filter off precipitate and wash with 75 ml of alcohol, 85% by volume. Dry filter paper and ignite in Pt dish. Add 10 ml of 0.1 *N* HCl, **XLII**, 5 and 6, and warm gently until all the lime dissolves. Cool, and titrate back with 0.1 *N* NaOH soln, using methyl orange indicator. The difference in ml divided by 10 represents the malic acid value of the sample. Previous to use, test reagents by a blank determination and apply any necessary corrections.

## SUGAR BEETS

## SUCROSE

125

*I. Alcohol Extraction Method<sup>43</sup>—Tentative*

Pass the sample (usually in the form of cossettes) thru a meat grinder fitted with a plate having  $\frac{1}{4}$ " perforations and mix thoroly. Weigh 26 g of the beet pulp and transfer to 100 ml flask with ca 50 ml of 90% alcohol and 3–5 ml of basic Pb acetate soln. Connect a reflux condenser to flask and place on boiling water bath for 10–15 min. Pour the whole into Soxhlet extractor, washing out flask with fresh portions of 90% alcohol. Connect the same 100 ml flask to the extractor and fit the latter with a return condenser. Add 90% alcohol until siphon is started and flask is ca  $\frac{3}{4}$  full. Place flask in covered water bath kept at a temp. that will allow the alcohol to boil freely. Continue extraction for 1–4 hours, or until a test of the alcohol in the extractor gives no color with alpha-naphthol soln when tested as follows: Introduce into a test tube a few drops of the alcohol coming from the extractor and add 4 or 5 drops of 20% alcoholic alpha-naphthol soln and 2 ml of H<sub>2</sub>O. Shake well, tip tube, allow 2–5 ml of colorless H<sub>2</sub>SO<sub>4</sub> to flow down side of tube, then hold tube upright. If sucrose is present, a color varying from faint to deep violet will be noted at junction of the two liquids. On shaking, the whole soln becomes blue violet color. This test is suitable for this work, but it must be remembered that other substances besides sucrose give this color reaction.

Remove flask from the water bath, transfer contents to 100 ml volumetric flask, cool to the standard temp., dilute to mark with 90% alcohol, shake, and filter, keeping funnel covered with watch-glass. Polarize in 200 mm tube.

Avoid evaporation and changes of temp. and use a minimum quantity of basic Pb acetate for clarification, 3 ml rather than 5 ml. By digesting the beet pulp with the alcohol before extraction, the time of extraction is greatly shortened, the pulp becomes thoroly impregnated with the alcohol, all the air is removed, and a good

extraction of the whole material is effected. If the pulp is fine and tends to clog the siphon, alcohol-washed cotton may be used as a plug in the extractor before adding the beet pulp, and a fine mesh screen may be placed over the pulp to keep the whole compact in the extractor.

## 126

*II. Hot Water Digestion Method I.<sup>44</sup>—Tentative*

Weigh 26 g of the prepared beet pulp, 125, and transfer with H<sub>2</sub>O to a wide-mouthed flask graduated to a content of 201.0 ml, the additional 1.0 ml representing an allowance for volume of marc and of Pb precipitate; add 5–10 ml of basic Pb acetate soln and sufficient H<sub>2</sub>O to produce a volume of 160–170 ml, and shake. Immerse flask in water bath at 80° and rotate at intervals. At end of 30 min. dilute to mark with H<sub>2</sub>O at 80° and continue digestion for 10 min. longer. Remove flask from water bath and allow it to cool to standard temp. Add sufficient strong acetic acid<sup>45</sup> to make the soln very slightly acid (generally less than 0.5 ml) and a few drops of ether to break the foam. Apply vacuum to withdraw retained air. Dilute to mark at 20°, mix thoroly, filter, and polarize in 400 mm tube.

## 127

*III. Hot Water Digestion Method II.<sup>46</sup>—Tentative*

Use Ni-plated sheet iron vessels, 11 cm high, 6 cm body diameter, and 4 cm mouth diameter, also stoppers covered with tinfoil to fit.

Weigh 26 g of the prepared beet pulp, 125, on a watch-glass (small enough to go into neck of beaker) and transfer to the metal beaker; add 177 ml of dilute basic Pb acetate soln (5 parts of basic Pb acetate soln, sp. gr. 1.25, to 100 parts of H<sub>2</sub>O); shake, and stopper lightly. Submerge beaker in water bath at 75–80° for 30 min., shaking intermittently. When all the air has been expelled (generally after 5 min.), tighten stopper. After 30 min., shake, cool to a standard temp., filter, add a drop of acetic acid to filtrate, and polarize in 400 mm tube. The reading is percentage of sugar in the beet pulp.

## SELECTED REFERENCES

- <sup>1</sup> Browne, Handbook of Sugar Analysis, 1912, p. 16.
- <sup>2</sup> J. Assoc. Official Agr. Chem., **8**, 255 (1925).
- <sup>3</sup> Ibid., **15**, 195 (1932); **18**, 83 (1935).
- <sup>4</sup> Ibid., **15**, 79 (1932); **16**, 81 (1933); **17**, 74 (1934).
- <sup>5</sup> Leach, 32nd Ann. Rpt. Mass. Board Health, 1900, p. 563; Leach-Winton, Food Inspection and Analysis, 4th ed., 1920, p. 654.
- <sup>6</sup> Z. Ver. deut. Zucker-Ind., **50**, (N. F. 37), 357 (1900); **63** (N. F. 50), 25 (1913); J. Ind. Eng. Chem., **5**, 167 (1913); J. Assoc. Official Agr. Chem., **18**, 162 (1935).
- <sup>7</sup> U. S. Bur. Standards Sci. Paper **268**, p. 67.
- <sup>8</sup> J. Assoc. Official Agr. Chem., **12**, 106 (1929).
- <sup>9</sup> J. Ind. Eng. Chem., **12**, 792 (1920).
- <sup>10</sup> J. Assoc. Official Agr. Chem., **16**, 78 (1933); **17**, 74 (1934).
- <sup>11</sup> Browne, Handbook of Sugar Analysis, 1912, p. 260; J. Assoc. Official Agr. Chem., **4**, 326 (1921).
- <sup>12</sup> J. Assoc. Official Agr. Chem., **8**, 256 (1925).
- <sup>13</sup> J. Ind. Eng. Chem., **16**, 562 (1924).
- <sup>14</sup> Ibid., 170.
- <sup>15</sup> Clark, The Determination of Hydrogen Ions, 1922, Fig. 8.
- <sup>16</sup> J. Assoc. Official Agr. Chem., **11**, 168 (1928); **16**, 79 (1933); **17**, 74 (1934).
- <sup>17</sup> Ibid., **8**, 258 (1925).
- <sup>18</sup> Ibid., 400.
- <sup>19</sup> Ibid., **9**, 33 (1926).
- <sup>20</sup> J. Ind. Eng. Chem., **13**, 793 (1921).
- <sup>21</sup> Z. Unters. Nahr. Genussm., **22**, 412 (1911).
- <sup>22</sup> Leach-Winton, Food Inspection and Analysis, 4th Ed., 1920, p. 652.
- <sup>23</sup> J. Soc. Chem. Ind., **42**, 32T (1923); J. Assoc. Official Agr. Chem., **9**, 35 (1926); **12**, 38 (1929).

- <sup>24</sup> J. Assoc. Official Agr. Chemists, **11**, 177 (1928); J. Biol. Chem., **23**, 81 (1915); J. Ind. Eng. Chem., **11**, 747 (1919).
- <sup>25</sup> J. Am. Chem. Soc., **28**, 663 (1906); **29**, 541 (1907).
- <sup>26</sup> J. Assoc. Official Agr. Chem., **12**, 28 (1929); **18**, 83 (1935).
- <sup>27</sup> J. Am. Chem. Soc., **57**, 845 (1935).
- <sup>28</sup> J. Research, Nat. Bur. Standards, **15**, 493 (1935); **19**, 691 (1937), RP1057.
- <sup>29</sup> Ibid., **22**, 697 (1939), RP1213.
- <sup>30</sup> J. Assoc. Official Agr. Chem., **15**, 79, 198 (1932).
- <sup>31</sup> J. Biol. Chem., **70**, 599 (1926).
- <sup>32</sup> U. S. Dept. Agr. Bur. Chem. Bulls., **110**, and **154**, Z. Nahr. Genussm., **18**, 625 (1909).
- <sup>33</sup> U. S. Dept. Agr. Bur. Chem. Bull., **110**, 60.
- <sup>34</sup> Ibid., **110** and **154**.
- <sup>35</sup> Ibid., **154**, p. 15; Ind. Eng. Chem., **17**, 612 (1925); J. Assoc. Official Agr. Chem., **15**, 78 (1932).
- <sup>36</sup> Z. Nahr. Genussm., **22**, 412 (1911).
- <sup>37</sup> Ibid., **19**, 72 (1910).
- <sup>38</sup> J. Am. Chem. Soc., **26**, 1523 (1904); J. Assoc. Official Agr. Chem., **15**, 79 (1932); **16**, 79 (1933); **17**, 73 (1934); **18**, 83 (1935); Trans. Roy. Soc. Can., 1919, Sec. 111, 221.
- <sup>39</sup> J. Assoc. Official Agr. Chem., **4**, 437 (1921); **16**, 80 (1933); **17**, 74 (1934).
- <sup>40</sup> J. Am. Chem. Soc., **28**, 1204 (1906); J. Assoc. Official Agr. Chem., **16**, 80 (1933); **17**, 74 (1934).
- <sup>41</sup> J. Assoc. Official Agr. Chem., **4**, 435 (1921); **16**, 80 (1933); **17**, 74 (1934); J. Ind. Eng. Chem., **5**, 741 (1913); **8**, 331 (1916).
- <sup>42</sup> J. Am. Chem. Soc., **30**, 1285 (1908).
- <sup>43</sup> U. S. Dept. Agr. Bur. Chem. Bull., **148**, p. 21.
- <sup>44</sup> Ibid., p. 18.
- <sup>45</sup> Deut. Zuckerind., 1897, 65.
- <sup>46</sup> U. S. Dept. Agr. Bur. Chem. Bull., **146**, p. 19.



## XXXV. VEGETABLES AND VEGETABLE PRODUCTS

1

### PHYSICAL EXAMINATION—TENTATIVE

(Applicable to canned products only.)

Note carefully external appearance of packages to detect presence of "leakers," "swells," or "springers." In general, the ends of sound tins of canned vegetables are slightly concave. If desired, the vacuum or pressure in the can may be taken by a gage designed for that purpose.

Measure distance from top of double seam to contents of can.

Note particularly odor of the vegetables, appearance of liquor or brine—whether clear or turbid—and condition of seams and inner walls of container, especially as to blackening and corrosion; note also general appearance, color, flavor, and size of the vegetables. In all instances the analyst should familiarize himself with normal appearance, odor, color, flavor, and other properties of the product under examination. Make careful macroscopic or microscopic examination for worm infestation, mold, dirt, or other evidence of decomposition or filth. If analysis of the gas in a bulged can is desired, the gas may be conveniently collected by means of the Doremus gas collector<sup>1</sup> or by similar apparatus.

2

### PREPARATION OF SAMPLE—OFFICIAL

(Applicable to canned products only.)

The preparation of the sample depends upon the character of the product and the determinations to be made. With samples composed of solid and liquid portions, proceed as follows: Weigh full can, then open, make physical measurements as directed under 1, pour entire contents on round sieve with a No. 8 standard screen (diameter of wire 0.84 mm and size of opening 2.38 mm).<sup>2</sup> Use a sieve 8" in diameter for a No. 3 or smaller can, and a sieve 12" in diameter for cans larger than No. 3. Without shifting the product, so incline sieve as to facilitate drainage of the liquid.

Allow material on sieve to drain 2 min., weigh either drained solids or free liquid direct, and reweigh dry empty can. From weights thus obtained determine percentage of liquid and of solid contents. If only solid portion is required for analysis or examination, thoroly grind drained vegetables in mortar or food chopper. If a composite of solid and liquid portion is required, thoroly grind entire contents of can in mortar or food chopper. In all cases, thoroly mix portion used and preserve balance in glass-stoppered containers. Unless the analysis is to be completed in a reasonably short time, determine moisture in a portion of the sample prepared as above, and to prevent decomposition dry remainder, grind, mix thoroly, and preserve in glass-stoppered containers. A second moisture determination is required in this procedure.

3

### TOTAL SOLIDS—TENTATIVE.—See 18.

4

### ASH—OFFICIAL.—See XXXIV, 9 or 10.

5

### SODIUM CHLORIDE—OFFICIAL

Determine Cl as directed under XII, 35 or 37. Express result in terms of NaCl.

6

### REDUCING SUGARS BEFORE INVERSION—OFFICIAL

Weigh 20 g of sample into 200 ml flask, dilute with ca 100 ml of H<sub>2</sub>O, clarify with a slight excess of neutral Pb acetate soln, dilute to mark, and filter. Remove excess

of Pb with anhydrous  $\text{Na}_2\text{SO}_4$  or with dry K oxalate. Filter, and determine reducing sugars as directed under XXXIV, 38. Express result as percentage of invert sugar.

7

## REDUCING SUGARS AFTER INVERSION—OFFICIAL

Transfer 50 ml of filtrate, 6, to 100 ml flask, add 5 ml of HCl, and let stand overnight, as directed under XXXIV, 24(c). Nearly neutralize with NaOH soln, cool, dilute to mark, and determine reducing sugars in an aliquot as directed under XXXIV, 38. Express result as percentage of invert sugar.

8

## SUCROSE—OFFICIAL.—See XXXIV, 29.

9

## TOTAL ACIDS—OFFICIAL

Proceed as directed under XXVI, 24, using 5 g of sample. Express result as number of ml of normal alkali required to neutralize 100 g of sample.

10

## VOLATILE ACIDS—OFFICIAL

Proceed as directed under XV, 24. Express result as percentage of acetic acid. 1 ml of 0.1 N alkali = 0.0060 g of acetic acid.

11

## PRESERVATIVES AND ARTIFICIAL SWEETENERS.—See XXXII.

12

## COLORING MATTERS.—See XXI.

13

## METALS.—See XXIX.

## 14 ALCOHOL-INSOLUBLE SOLIDS IN CANNED PEAS AND CANNED DRIED PEAS—OFFICIAL

Pour sample on an 8-mesh screen, using an 8" screen for containers of less than 3 lbs. net weight, and a 12" screen for larger quantities. Spread peas evenly and allow to drain. Transfer peas to white pan and remove any foreign material. Add a volume of  $\text{H}_2\text{O}$  equal to double the volume of original sample.

Pour peas back on screen, spreading evenly; tilt screen as much as possible without shifting peas; and drain for 2 min. With a cloth wipe surplus moisture from lower surface of screen. Grind drained peas in food chopper until the cotyledons are reduced to a smooth homogeneous paste, stir, and weigh 20 g of the ground material into 600 ml beaker. Add 300 ml of 80% alcohol (b $\&$  volume), stir, cover beaker, and bring to boil. Simmer slowly 30 min.

Fit into Büchner funnel a filter paper of appropriate size, previously prepared by drying in flat-bottomed dish 2 hours at temp. of boiling  $\text{H}_2\text{O}$ , covering with tight-fitting cover, cooling in desiccator, and weighing at once. Apply suction and transfer contents of beaker to the Büchner funnel, in such manner as to avoid running over edge of paper. Suck dry and wash material on filter with the 80% alcohol until washings are clear and colorless.

Transfer filter paper and alcohol-insoluble solids to the dish used in preparation of filter paper, dry uncovered for 2 hours at temp. of boiling  $\text{H}_2\text{O}$ , place cover on dish, cool in desiccator, and weigh at once. From this weight deduct weight of dish, cover, and paper. Calculate this weight to percentage.

15 FIELD CORN IN CANNED MIXTURES OF FIELD AND SWEET CORN<sup>4</sup>—TENTATIVE

Empty contents of a No. 2 can, or the representative equivalent portion of a larger can, into large beaker and remove liquor and debris from fragments of kernels by flotation with cold  $\text{H}_2\text{O}$ . Place upon a flat plate all kernels to which the outer seed coat is still attached, mix thoroly, and quarter to ca 400 pieces. Harden selected pieces in alcohol and quarter again to obtain ca 100 fragments. Cut each fragment thru with a section razor or knife and avoid contamination of fragments with

dextrin by washing and drying the instrument after each cut. With a dissecting needle remove portion ca  $\frac{1}{16}$ " in diameter from uncontaminated interior of each kernel and place pieces in separate depressions of white spot plate. Cover each piece with freshly prepared I stain (0.2 g of I, 1.5 g of KI in 100 ml of  $H_2O$ ) and allow to stand 10 min. A brown cloud will disseminate from the portions of sweet corn due to dextrin, while the soln surrounding the field corn will remain clear and the portion will be blue black and sharply outlined. Crush the field corn portions to insure absence of dextrin and count those found to contain none. Use care in interpreting results because kernels of immature sweet corn do not contain enough dextrin to produce the dense brown coloration characteristic of more mature sweet corn. In case of doubt, report as field corn only those kernels having a firm texture and showing no brown coloration with I soln on 2 confirmatory tests. Calculate percentage of field corn from total number of kernels examined.

### TOMATO PRODUCTS<sup>5</sup>

(Tomato catsup, pulp, purée, sauce, and paste.)

16

#### PREPARATION OF SAMPLE—OFFICIAL

Shake unopened container thoroly to incorporate any sediment. Transfer entire contents to large glass or porcelain dish and mix thoroly, continuing the stirring for at least 1 min. Transfer well-mixed sample to glass-stoppered container and shake or stir thoroly each time before removing portions for analysis.

17

#### SPECIFIC GRAVITY<sup>6</sup>—TENTATIVE

Determine the sp. gr. at 20/20°, using a National Canners Association sp. gr. bottle. Clean and calibrate the bottle at 20° as directed under XIV, 3, but since the bottle is not provided with a cap, strike off excess  $H_2O$  with a straight edge, wipe bottle dry, and weigh immediately. Cool sample to 16–18°, fill flask with the pulp, and place it in a centrifuge with a suitable counterpoise in the other receptacle. Whirl for 1 min. at a speed of ca 1000 r.p.m. Add sufficient pulp to fill flask to top and whirl centrifuge again. Remove flask and take temp. of pulp, inserting thermometer so that no air is introduced. When temp. is just 20°, remove thermometer, add sufficient pulp at same temp. to have flask slightly overfull, and strike off even with a straight edge. Clean outside of flask and weigh at once to nearest 0.01 g. Sp. gr. = weight of pulp in flask ÷ weight of  $H_2O$  at 20° that flask holds.

18

#### TOTAL SOLIDS—TENTATIVE

(Applicable to canned products only.)

Weigh into flat-bottomed dish a portion of sample of such size that the dry residue will not be less than 9 mg nor more than 12 mg per sq. cm of drying surface. Distribute thinly in even layer over bottom of dish, diluting with  $H_2O$  if necessary to facilitate distribution. Place in vacuum oven at 70° with release cock left partly open so that the degree of vacuum does not exceed 450 mm of Hg and the moisture evolved is carried off rapidly. Dry the air admitted thru release cock by bubbling thru  $H_2SO_4$ . At the end of an hour examine dishes and remove from oven any in which material has reached apparent dryness. Continue this removal of dishes with dried material at subsequent half-hour intervals. After material in all dishes has reached apparent dryness return dishes to oven, nearly close release cock so that ca 2 bubbles of air per second are admitted thru the  $H_2SO_4$  and dry at 70° for 4 hours at a pressure not exceeding 100 mm.

19

## INSOLUBLE SOLIDS—TENTATIVE

Wash 20 g of sample repeatedly with hot  $H_2O$ , centrifuging after each addition of  $H_2O$  and pouring the clear, supernatant liquid thru weighed paper filter on Büchner funnel. The filter used is one of two such papers dried 2 hours at  $100^\circ$  and weighed in covered dish. The second paper is used, if necessary, when first paper becomes clogged. After 4 or 5 washings transfer remaining insoluble matter to filter, dry in covered dish for 2 hours at  $100^\circ$ , cool in desiccator, and weigh.

20

## SOLUBLE SOLIDS—TENTATIVE

Percentage of total solids—percentage of insoluble solids = percentage of soluble solids.

21

## SAND—TENTATIVE

Weigh 100 g of well-mixed sample into 2–3 liter beaker, nearly fill beaker with  $H_2O$  and mix contents thoroly. Allow to stand 5 min. and decant supernatant liquid into second beaker. Refill first beaker with  $H_2O$  and again mix contents. After 5 min. decant the second beaker into a third and the first into the second; refill and again mix the first. Continue this operation, decanting from the third beaker into the sink until the lighter material is washed from the sample. Collect the sand from the 3 beakers on weighed Gooch crucible, dry, ignite, and weigh.

Under "Sand" only the figure obtained by above method should be reported. The results obtained by the determination of ash insoluble in  $HCl$  are not applicable to the determination of sand, as the sand is so unevenly distributed that reliable results can be obtained only by taking a larger sample than is possible in the determination of ash.

22

## ASH—OFFICIAL.—See XXXIV, 9 or 10.

23

## ALKALINITY OF THE ASH—OFFICIAL

Proceed as directed under XXVI, 10. Express result as number of ml of normal acid required to neutralize the ash from 100 g of the sample.

24

## SODIUM CHLORIDE

*Method I—Official*

Proceed as directed under XII, 35 or 37, using a  $HNO_3$  soln of the ash (cf. XII, 34). Calculate and report result as percentage of  $NaCl$ .

25

*Method II (Rapid Method)<sup>7</sup>—Tentative*

Weigh 5 g of material and transfer with 80%  $C_2H_5OH$  to a 100 ml volumetric flask. Add 80%  $C_2H_5OH$  to give a volume of ca 50 ml. Shake well to get all tomato material into suspension. Add 1 ml of  $HNO_3$  and by means of pipet add an excess of 0.1  $N$   $AgNO_3$ . Make to 100 ml with alcohol. Transfer mixture to centrifuge bottle and centrifuge at ca 1800 r.p.m. 5 min. Pipet 50 ml of supernatant liquid into a 300 ml Erlenmeyer flask, add 2 ml of saturated soln of ferric ammonium sulfate and 2 ml of  $HNO_3$ , and titrate to permanent light brown color with 0.1  $N$   $NH_4CNS$  soln. Divide number of ml of 0.1  $N$   $AgNO_3$  used by 2 and subtract the number of ml's of  $NH_4CNS$  used. Multiply difference by 0.005843 to obtain weight of chlorides present expressed as grams of  $NaCl$ . Divide by 2.5 and multiply by 100 to calculate percentage of salt present.

26

## SUGARS.—See 6, 7, and 8.

27

## TOTAL ACIDS—OFFICIAL

Proceed as directed under XXVI, 24, using 5 g of sample. Express result as percentage of anhydrous citric acid. 1 ml of 0.1 *N* alkali = 0.0064 g of anhydrous citric acid.

28

## VOLATILE ACIDITY—OFFICIAL

Proceed as directed under XV, 24, using 25 g of sample, increasing quantity of H<sub>2</sub>O used for distillation, and collecting correspondingly larger quantity of distillate. Express result as percentage of acetic acid. 1 ml of 0.1 *N* alkali = 0.0060 g of acetic acid.

29

## FIXED ACIDITY—OFFICIAL

Multiply percentage of volatile acids, 28, by 1.067 and subtract product from percentage of total acids, 27, to obtain percentage of fixed acids as citric acid.

## MICROANALYSIS OF TOMATO PULP, PURÉE, SAUCE, PASTE—OFFICIAL

30

## APPARATUS

(a) *Compound microscope*.—Equipped with good objectives and oculars, giving magnifications of ca 90, 180, and 500 diameters. For convenience of use the lenses should be adjusted so as to be parfocal. A mechanical stage is highly desirable. It is essential that the combination giving the low magnification be capable of adjustment to give an area of the field of view of 1.5 sq. mm (a circle whose diameter is 1.382 mm). With the higher powers the working distances must be ample to allow free use of the blood-counting cell.

(b) *Drop-in cross-ruled disk*.<sup>9</sup>—For estimating lengths of mold filaments, an ocular drop-in disk cross-ruled in sixths of the ocular diaphragm opening is desirable.

(c) *Blood counting cell*.—Ruled in the Thoma or the old Neubauer system of rulings. The so-called "improved" system of Neubauer is not suitable for this purpose.

(d) *Howard mold-counting cell*.<sup>10</sup>—Constructed like a blood-counting cell but with unruled central disk ca 19 mm in diameter.

31

## MOLDS

In making mold counts of tomato products, use the material as is except that clean, mold-free gum may be added to thin products to assist in making more uniform mounts; in the case of tomato products of such heavy consistency as to make observation of the mold filaments difficult, mix H<sub>2</sub>O to make total tomato solids of diluted product 8.37 to 9.37%. If the product contains salt or other substance that increases solids content materially, take this into consideration in making dilution.

Clean the special Howard cell so that Newton's rings are produced between slide and cover-glass. Remove cover and place small drop of the well-mixed sample upon central disk; using knife blade or scalpel, spread drop evenly over disk, and cover with the glass so as to give an even spread.

It is of the utmost importance that the drop be taken from a thoroly mixed sample and spread evenly over the slide disk. Otherwise, when cover slip is put in place the insoluble material, and consequently the molds, may be more abundant at center of mount. Avoid using a drop that is much greater than is sufficient to fill space between center disk and cover slip. Discard any mount showing uneven distribution, absence of Newton's rings, or liquid that has been drawn across moat and under cover-glass.

Place slide under microscope and examine with such adjustment that each field of view covers 1.5 sq. mm. This area, which is of vital importance, may frequently

be obtained by so adjusting the draw-tube that the diameter of the field becomes 1.382 mm. When such adjustment is not possible, it is sometimes necessary to have a mechanic make an accessory drop-in ocular diaphragm with the aperture accurately cut to necessary size. The diameter of area of field of view can be determined by use of a stage micrometer, or by employing the rulings on the blood-counting cell. In order to use latter method it is necessary to bear in mind that a square whose diagonal is 1.382 mm has sides of ca 0.977 mm. Hence the mm scale on the blood-counting cell can be used by such adjustment that the circle of the field of view cuts off necessary amount from each corner of the mm-ruled square. When the instrument is properly adjusted, the quantity of liquid examined per field is 0.15 cmm (0.00015 ml).

From each of two or more mounts examine at least 25 fields taken in such manner as to be representative of all sections of the mount. Observe each field, noting presence or absence of mold filaments and recording result as positive or as negative, as case may be. No field should be considered positive unless the aggregate length of not more than three of the filaments present exceeds ca  $\frac{1}{3}$  of diameter of field. Calculate proportion of positive fields from results of examination of all observed fields and report as percentage of fields containing mold filaments.

## 32

## YEASTS AND SPORES

Fill graduated cylinder with H<sub>2</sub>O to 20 ml mark and add sample till level of mixture reaches 30 ml mark. Close graduate, or pour contents into Erlenmeyer flask, and shake vigorously 15–20 seconds. To assure thoroughness the mixture should not fill more than  $\frac{2}{3}$  of container in which shaking is performed. For tomato sauce or pastes, or products running high in number of organisms or of heavy consistency, use 80 ml of H<sub>2</sub>O with 10 ml or 10 g of sample. In the case of exceptionally thick or dry pastes, it may be necessary to make even greater dilution.

Pour mixture into beaker. Thoroughly clean blood counting cell so as to give good Newton's rings. Stir thoroughly contents of beaker with scalpel or knife blade and after allowing to stand 3–5 seconds remove a small drop, place it upon the central disk of the blood-counting cell, and cover immediately with cover-glass. Discard any mount showing uneven distribution, absence of Newton's rings, or liquid that has been drawn across moat and under cover-glass. Allow slide to stand not less than 10 min. before beginning to make the count. It is customary to make the count with a magnification of 180–220 diameters.

Count number of yeasts and mold spores on  $\frac{1}{2}$  of ruled squares on disk (this amounts to counting the number in 8 of the blocks, each of which contains 25 of the small ruled squares). Total number thus obtained equals number of organisms in 1/60 cmm (1/60,000 ml of original product) if a dilution of 1 part of sample with 2 parts of H<sub>2</sub>O is used. If a dilution of 1 part of sample with 8 parts of H<sub>2</sub>O is used, multiply the number by 3. In making the counts, the analyst should avoid counting twice the organisms that rest on a boundary line between two adjacent squares.

## 33

## BACTERIA

Determine number of rod-shaped bacteria from mounted sample used in 32, but before examination allow sample to stand not less than 15 min. after mounting. Use magnification of ca 500 diameters.

Count, and record number of bacteria having a length greater than  $1\frac{1}{2}$  times their width in an area including 5 of the small squares. Count number in 5 such areas, preferably 1 from near each corner of ruled portion of slide and 1 from near the center. Determine total number of rod-shaped bacteria in the 5 areas and multiply by 480,000. This gives number of this type of bacteria per ml. If a dilution of 1 part

of sample with 8 parts of  $H_2O$ , instead of 1 part of sample with 2 parts of the  $H_2O$ , is used in making up the sample, then total count obtained in the 5 areas must be multiplied by 1,440,000. The bacteria sometimes exhibit slight motion and thus may present momentarily an end instead of a side view. For this reason it is necessary to keep them under observation long enough to establish their true character. Thus far it has proved impracticable to count the micrococci present as they are likely to be confused with other bodies frequently present in such products.

#### SELECTED REFERENCES

- <sup>1</sup> J. Am. Chem. Soc., 19, 733 (1897); Leach, Food Inspection and Analysis, 4th ed., 1920, p. 970.
- <sup>2</sup> U. S. Bur. Standards Specifications for Sieves, U. S. Standard Sieve Series.
- <sup>3</sup> J. Assoc. Official Agr. Chem., 21, 90 (1938); 22, 87 (1939).
- <sup>4</sup> Ibid., 12, 39 (1929); 15, 167 (1932).
- <sup>5</sup> U. S. Dept. Agr. Bur. Chem. Bull., 162, p. 124.
- <sup>6</sup> N.C.A. Bull. 21 L, p. 136.
- <sup>7</sup> J. Assoc. Official Agr. Chem., 22, 765 (1939).
- <sup>8</sup> J. Ind. Eng. Chem., 7, 603 (1915); U. S. Dept. Agr. Bull., 581; J. Assoc. Official Agr. Chem., 3, 453 (1920); 5, 226 (1921); New York Agr. Exp. Sta., Geneva, N. Y., Tech. Bull., 91.
- <sup>9</sup> J. Assoc. Official Agr. Chem., 6, 50 (1922).
- <sup>10</sup> U. S. Dept. Agr. Bur. Chem. Circ. 68, p. 4.

---

#### XXXVI. VITAMINS

- 1                    *Vitamin D Milk—Tentative.—See XXII, 41.*
- 2                    *Vitamin D for Poultry—Tentative.—See XXVII, 65.*

## XXXVII. WATERS, BRINE, AND SALT

### WATERS

#### POTABLE WATER

##### TURBIDITY—OFFICIAL

1

##### REAGENTS

(a) *Standard turbidity soln.*—Mix 1 g of elutriated fullers' earth, previously dried and sifted thru 200-mesh sieve, with  $H_2O$  and dilute to 1 liter. This stock mixture has a turbidity of 1000. Check the stock soln with a turbidimeter equipped with either candle or electric light.

(b) *Turbidity standards.*—Prepared by dilution of (a).

2

##### DETERMINATION

If the turbidity is less than 100, which prohibits the use of the turbidimeter, determine by direct comparison with turbidity standards contained in bottles of clear white glass.

##### COLOR—OFFICIAL

3

##### REAGENTS

(a) *Standard color soln.*—Dissolve 1.245 g of potassium chloroplatinate ( $PtCl_4 \cdot 2KCl$ ) containing 0.5 g of Pt, and 1 g of  $CoCl_2 \cdot 6H_2O$  containing 0.25 g of Co, in 100 ml of  $H_2O$ ; add 100 ml of  $HCl$  and dilute to 1 liter with  $H_2O$ . This stock mixture has a color of 500.

(b) *Color standards.*—Prepared by dilution of (a).

4

##### DETERMINATION

Compare color of the sample, freed from suspended matter, with the standards in 50 ml Nessler tubes of clear white glass.

5

##### ODOR—OFFICIAL

Shake the vessel containing sample and note odor. Heat a portion of sample to incipient boiling and note odor.

6

##### TOTAL SOLIDS—OFFICIAL

Thoroughly shake the vessel containing sample and pipet 100 ml of the unfiltered  $H_2O$  into weighed Pt dish. If sample contains much suspended matter, shake, pour rapidly into 100 ml measuring cylinder, and transfer without delay to weighed Pt dish. Evaporate to dryness and heat to constant weight at  $100^\circ$ .

7

##### SOLIDS IN SOLUTION—OFFICIAL

Allow sample to stand until all sediment has settled and filter if necessary to secure a perfectly clear liquid. Occasionally a clear filtrate can be obtained only by the use of alumina cream, but this should be avoided if possible. Evaporate 100–250 ml to dryness in weighed Pt dish. Heat to constant weight at  $100^\circ$ .

8

##### IGNITED RESIDUE—OFFICIAL

Ignite residue from 6 until dish shows a dull red glow and ash is white or nearly so.



Note any odor or change in color produced during ignition. Record weight of ignited residue and calculate loss on ignition.

9

## SUSPENDED MATTER—OFFICIAL

(1) Subtract the value for solids in soln, 7, from the value for total solids, 6; or, (2) filter a suitable measured volume of sample thru dry weighed Gooch crucible containing an asbestos mat. Dry crucible and contents at 100°, cool, and weigh.

## NITROGEN IN THE FORM OF FREE AND ALBUMINOID AMMONIA

*Method I.—Official*

(For waters that do not contain hydrogen sulfide.)

10

## REAGENTS

(a) *Nessler's reagent*.—Dissolve 143 g of NaOH in 950 ml of H<sub>2</sub>O and filter thru asbestos. Add 50 g of red HgI<sub>2</sub> to filtrate and dilute with H<sub>2</sub>O to 1 liter. Mix thoroly, allow to settle, and use supernatant liquid.

(b) *Alkaline potassium permanganate soln*.—Dissolve 143 g of NaOH and 8 g of KMnO<sub>4</sub> in H<sub>2</sub>O and dilute to 1 liter.

(c) *Standard ammonium chloride soln*.—Dissolve 3.818 g of reagent-grade NH<sub>4</sub>Cl in ammonia-free H<sub>2</sub>O and dilute to 1 liter. Then dilute 10 ml of this soln to 1 liter (1 ml = 0.01 mg of N, or 0.0128 mg of NH<sub>4</sub>).

11

## DETERMINATION

Connect a flask of 1000 ml capacity with upright bulb condenser by means of a half-inch glass tube and soft rubber stopper or recently extracted cork stopper. Place in flask 5 ml of a saturated soln of Na<sub>2</sub>CO<sub>3</sub> and 500 ml of NH<sub>3</sub>-free H<sub>2</sub>O. Distil and collect the distillate into 50 ml Nessler tubes until no further traces of NH<sub>3</sub> are indicated on addition of 2 ml of the Nessler reagent to 50 ml of the distillate. Continue distillation until volume of soln in flask has been reduced to ca 200 ml. Cool slightly, add 500 ml of sample, and distil, at rate of ca 1 tubeful in 10 min., into 50 ml Nessler tubes until NH<sub>3</sub> ceases to be given off (4 or 5 tubes are usually sufficient). Add 2 ml of the Nessler reagent to each tube and let stand 10 min. From a small buret measure definite quantities of the NH<sub>4</sub>Cl soln into Nessler tubes. Dilute to 50 ml with NH<sub>3</sub>-free H<sub>2</sub>O, add 2 ml of the Nessler reagent, and compare depth of color with Nesslerized distillate. Report as mg per liter of N in the form of free NH<sub>3</sub>. Cool flask and add 50 ml of the alkaline permanganate recently boiled. Distil and Nesslerize as directed above.

*Method II.—Official*

(For waters containing sulfur.)<sup>2</sup>

12

## REAGENTS

(a) *Sulfuric acid soln*.—Dilute 7 ml of H<sub>2</sub>SO<sub>4</sub> (free from NH<sub>4</sub>-salts) to 500 ml.

(b) *Sodium carbonate soln*.—Dissolve 66 g of anhydrous Na<sub>2</sub>CO<sub>3</sub> or 179 g of Na<sub>2</sub>CO<sub>3</sub> · 10H<sub>2</sub>O in H<sub>2</sub>O and dilute to 250 ml.

The other reagents and solns used are described under 10.

13

## DETERMINATION

Place 500 ml of sample in a casserole, add 30 ml excess of the H<sub>2</sub>SO<sub>4</sub> soln, and boil carefully until free from sulfide (ca 20 min.). Place ca 300 ml of H<sub>2</sub>O and 8 ml of the Na<sub>2</sub>CO<sub>3</sub> soln in a distilling flask connected as described under 11 and distil until

free from  $\text{NH}_3$ . Cool, add the cooled sample, which is now free from sulfide, and proceed with the distillation, addition of alkaline permanganate soln, etc., as directed under 11.

## NITROGEN IN THE FORM OF NITRITE—OFFICIAL

14

## REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 1 g of sulfanilic acid in hot  $\text{H}_2\text{O}$ , cool and dilute to 100 ml.

(b) *Alpha-naphthylamine hydrochloride soln.*—Boil 0.5 g of the salt with 100 ml of  $\text{H}_2\text{O}$ , kept at constant volume, for 10 min.

(c) *Standard nitrite soln.*—Dissolve 1.1 g of  $\text{AgNO}_2$  in  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ , precipitate the Ag with NaCl soln, dilute to 1 liter, mix, and allow to settle. Dilute 100 ml to 1 liter and then 10 ml of this soln to 1 liter, using in each case  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ . 1 ml of the last soln = 0.0001 mg of N (0.0003 mg of  $\text{NO}_2$ ).

15

## DETERMINATION

Place 100 ml of sample in 100 ml Nessler tube and add HCl dropwise until sample shows acid reaction to litmus paper. Add 1 ml of the sulfanilic acid and 1 ml of the alpha-naphthylamine hydrochloride soln, and thoroly mix. Set aside for 30 min. with other Nessler tubes containing known quantities of the  $\text{NO}_2$  soln made up to 100 ml with  $\text{NO}_2$ -free  $\text{H}_2\text{O}$  and acidified with HCl, the sulfanilic acid, and the alpha-naphthylamine hydrochloride solns, in the same manner as the sample. Determine quantity of N by comparing the depth of pink color in the known and unknown solns. Record result as N.

## NITROGEN IN THE FORM OF NITRATE\*

## I. Phenoldisulfonic Acid Method—Official

(For water of low chlorine content.)

16

## REAGENTS

(a) *Phenoldisulfonic acid soln.*—Dissolve 25 g of pure white phenol in 150 ml of  $\text{H}_2\text{SO}_4$ , add 75 ml of fuming  $\text{H}_2\text{SO}_4$  (13–15%  $\text{SO}_3$ ), and heat at  $100^\circ$  for 2 hours.

(b) *Standard nitrate soln.*—Dissolve 0.607 g of pure  $\text{NaNO}_3$  in 1 liter of  $\text{NO}_3$ -free  $\text{H}_2\text{O}$ . Evaporate 50 ml of this soln to dryness in a porcelain dish; when cool, treat with 2 ml of the phenoldisulfonic acid soln, grind, and stir with a glass rod to insure intimate contact; and dilute to 500 ml. 1 ml = 0.01 mg of N (0.04 mg of  $\text{NO}_3$ ). (This soln is permanent.) Prepare standards for comparison by adding  $\text{NH}_4\text{OH}$  to measured volumes of the standard soln in 100 ml Nessler tubes.

(c) *Standard silver sulfate soln.*—Dissolve 4.397 g of  $\text{Ag}_2\text{SO}_4$ , free from  $\text{NO}_3$ , in 1 liter of  $\text{H}_2\text{O}$ . 1 ml = 1 mg of Cl.

17

## DETERMINATION

To 100 ml of sample, or a quantity that contains 0.05 mg or less of N, add the standard  $\text{Ag}_2\text{SO}_4$  soln, precipitating all but ca 0.5 mg of the Cl. Heat to boiling and allow to settle, or add a little alumina cream, filter, and wash with small quantities of hot  $\text{H}_2\text{O}$ . Evaporate filtrate to dryness in porcelain dish on steam bath; when cool, treat with 2 ml of the phenoldisulfonic acid soln as directed under 16(b). Dilute with  $\text{H}_2\text{O}$  and add slowly  $\text{NH}_4\text{OH}$  until the maximum color is developed. Filter if necessary, transfer to colorimetric cylinder, and compare with the standards in the usual manner. Record result as N.

II. Reduction Method<sup>4</sup>—Official

(For water of high chlorine content.)

18

## REAGENTS

(a) *Aluminum foil*.—Should be purest obtainable. Cut into strips ca 10 cm long, weighing ca 0.5 g.

(b) *Sodium hydroxide soln*.—Dissolve 250 g of pure NaOH in 1250 ml of H<sub>2</sub>O. Add 2 or 3 strips of the Al foil and let stand ca 12 hours. Concentrate the soln to 1 liter by boiling.

19

## DETERMINATION

To 100 ml of sample, or a quantity that contains 0.1 mg or less of N as NO<sub>3</sub>, in 300 ml casserole, add 2 ml of the NaOH soln and concentrate by boiling to ca  $\frac{1}{3}$  original volume. Transfer to 100 ml test tube, using N-free H<sub>2</sub>O, and dilute, if necessary, to volume of ca 75 ml. Prepare a blank (preferably several blanks, since the N impurity in Al is often distributed unevenly) by placing ca 75 ml of N-free H<sub>2</sub>O and 2 ml of the NaOH soln in 100 ml test tube. Place a strip of the Al foil in each tube. Close ends of test tubes with rubber stoppers connected by means of bent glass tubes with other test tubes containing ca 50 ml of slightly acidified NH<sub>3</sub>-free H<sub>2</sub>O. (These latter tubes serve as traps to prevent escape of NH<sub>3</sub> and at the same time permit the free evolution of H.) Allow sample and blank to stand at room temp. for 12 hours or until reduction is complete. Nesslerize the traps. If high in NH<sub>3</sub>, indicating frothing over of sample, discard determination. Disregard the traps if they contain only 0.01–0.02 mg of N as NH<sub>3</sub> each. Transfer sample and blank to distillation flasks, using 250 ml of NH<sub>3</sub>-free H<sub>2</sub>O for each; distil, Nesslerize, and compare with standards as in determination of free NH<sub>3</sub>, 11. Subtract quantity of N found in blank from that found in sample. Calculate to mg per liter of N.

## CHLORIDE—OFFICIAL

20

## REAGENTS

(a) *Potassium chromate indicator*.—Dissolve 5 g of K<sub>2</sub>CrO<sub>4</sub> in H<sub>2</sub>O, add a saturated soln of AgNO<sub>3</sub> until a slight permanent red precipitate is produced, filter, and dilute to 100 ml.

(b) *Standard silver nitrate soln*.—Dissolve 4.791 g of AgNO<sub>3</sub> in H<sub>2</sub>O and dilute to 1 liter. 1 ml = 1 mg of Cl. Check by titration against a standardized soln of NaCl.

21

## DETERMINATION

To 100 ml of sample add a few drops of phenolphthalein indicator. If pink color appears, titrate the CO<sub>3</sub> thus indicated to HCO<sub>3</sub> with 0.05 N H<sub>2</sub>SO<sub>4</sub>. If sample is acid to methyl orange, add 0.05 N Na<sub>2</sub>CO<sub>3</sub> to neutralize acidity. Add 1 ml of the K<sub>2</sub>CrO<sub>4</sub> indicator and titrate with the standard AgNO<sub>3</sub> soln. Correct for the quantity of AgNO<sub>3</sub> soln necessary to give, in 100 ml of Cl-free H<sub>2</sub>O with 1 ml of the chromate, the shade obtained at the end of the titration of the sample. (If iodides and bromides are found in interfering quantities, make equivalent correction.)

If chlorides are present in very small quantities, concentrate 500 or 1000 ml in a porcelain dish to 100 ml, rub down sides of dish carefully, add 1 ml of the K<sub>2</sub>CrO<sub>4</sub> indicator, and titrate with the standard AgNO<sub>3</sub> soln. If sufficient chlorides are present in 100 ml of the H<sub>2</sub>O to consume more than 25 ml of the standard AgNO<sub>3</sub> soln, determine by precipitation and weigh the AgCl as directed under XII, 35.

## FLUORIDES—TENTATIVE

22

## REAGENTS

(a) *Sodium fluoride soln.*—Dissolve 2.22 g of NaF (purity at least 98%) in 1 liter of H<sub>2</sub>O. (This soln contains 1 mg of F per ml.)

(b) *Standard sodium fluoride soln.*—Dilute 10 ml of stock soln (a) to 1 liter (1 ml = 0.01 mg of F.)

(c) *Thorium nitrate soln.*—Dissolve 0.25 g of Th(NO<sub>3</sub>)<sub>4</sub>·12H<sub>2</sub>O in 1 liter of H<sub>2</sub>O.

(d) *Alizarin red indicator.*—Make a 0.01% H<sub>2</sub>O soln of Na alizarin sulfonate.

(e) *Hydrochloric acid.*—(1+249.) Dilute 4 ml of HCl to 1 liter.

(f) *Sodium hydroxide soln.*—Approximately 0.05 N (2 g of NaOH per liter).

23

## APPARATUS

(a) *Claissen flask.*—Capacity 250 ml.

(b) *Nessler tubes.*—6 long-form 50 ml tubes with double optically-plane disks fused to the tubes. Match tubes for length and test for optical similarity by filling to mark with 40 ml of H<sub>2</sub>O, 1 ml of the indicator, and 2 ml of the HCl. Then to 1 tube add such a quantity of the Th(NO<sub>3</sub>)<sub>4</sub> soln that, after mixing, the color barely changes to faint pink. Note quantity of Th(NO<sub>3</sub>)<sub>4</sub> soln used. Add same quantity of Th(NO<sub>3</sub>)<sub>4</sub> to each of remaining 5 tubes. Reject tubes showing detectable differences in shade or intensity.

24

## PREPARATION OF SAMPLE

Place 100 ml of sample in porcelain dish, make alkaline to phenolphthalein with the NaOH (avoid excess), and evaporate to 20 ml. During evaporation keep sample alkaline by adding small quantities of the NaOH soln from time to time. Transfer 20 ml of the evaporated sample to Claissen flask containing pebbles or boiling tube (which have been rinsed with boiling 10% NaOH to eliminate all traces of gelatinous silica accumulating in flask).

Place the flask containing sample on an asbestos board (6"×6"× $\frac{1}{4}$ " with 1" hole in center) over burner adjusted for medium size flame. Close the straight neck of the flask with a two-holed rubber stopper thru which pass a thermometer and the stem of a small separatory funnel, the outlet of which is constricted to 2 mm diameter. (The thermometer and the outlet tube of the funnel should extend almost to the bottom of the flask.) Close the other neck of flask with a solid rubber stopper. Connect flask with water condenser, add 20 ml of 60% HClO<sub>4</sub> to flask via the evaporating dish and funnel, and distil at 132 ± 3°. Collect nearly 200 ml of distillate. Make to volume (200 ml) and mix well. To determine acidity place 40 ml of distillate in Nessler tube, add 1 ml of the indicator, and note number of ml of the NaOH required for neutralization.

25

## DETERMINATION

Prepare one standard and one or more sample tubes as follows:

(a) *Sample tube.*—To sample tube containing 40 ml of distillate add 1 ml of the indicator and such quantity of the HCl that the total amount of acid in tube (acidity previously determined plus quantity of HCl added) equals 2 ml of the HCl. If in the preliminary acidity determination it is found that the 40 ml distillate requires more than 2 ml of the NaOH soln for neutralization, do not add the HCl to the sample tube, but add to the standard tube the same quantity of acid as was found present in the sample tube. If 40 ml of the distillate requires more than 5 ml of 0.05 N NaOH, repeat the distillation under conditions favorable to low acidity.

Add exactly the same quantity of  $\text{Th}(\text{NO}_3)_4$  that was added to the standard tube. Mix thoroly.

(b) *Standard tube*.—To standard tube containing 40 ml of  $\text{H}_2\text{O}$  add 1 ml of the indicator and 2 ml of the  $\text{HCl}$ . From a 10 ml buret graduated in 0.05 ml divisions add the  $\text{Th}(\text{NO}_3)_4$  soln until a faint pink color appears. Note volume of  $\text{Th}(\text{NO}_3)_4$  used. Mix thoroly. Into the standard tube (now more highly colored than sample tube) add the standard soln from a 10 ml buret until the color matches that of the sample tube. Make contents of both standard and sample tubes to same volume. Mix the soln in each tube with a long stirring rod and allow all air bubbles to escape before making color comparisons. Check the end point by adding 1–2 drops of the  $\text{NaF}$  soln to the standard tube. A distinct color change should develop.

## 26

## CALCULATION

$$\frac{\text{ml of NaF soln} \times \text{ml of total distillate} \times 10}{\text{ml aliquot titrated} \times \text{wt. of sample taken}} = F \text{ (p.p.m.)}$$

EXAMPLE: A 100 ml sample, evaporated and distilled to 200 ml of which a 40 ml aliquot corresponds to 5 ml of the  $\text{NaF}$  soln, gives:

$$\frac{5 \times 200 \times 10}{40 \times 100} = 2.5 F \text{ (p.p.m.)}$$

## OXYGEN REQUIRED

(By decomposition of organic matter present.)

*Method I.—Official*

## 27

## REAGENTS

(a) *Standard potassium permanganate soln*.—Dissolve 0.395 g of  $\text{KMnO}_4$  in 1 liter of  $\text{H}_2\text{O}$ . Each ml has 0.1 mg of O available for oxidation.

(b) *Standard oxalic acid soln*.—Dissolve 0.788 g of crystallized oxalic acid in 1 liter of  $\text{H}_2\text{O}$ .

Determine value of the oxalic acid in terms of the permanganate by boiling 10 ml of the oxalic acid and 200 ml of redistilled  $\text{H}_2\text{O}$  (prepared by treating distilled  $\text{H}_2\text{O}$  with alkaline permanganate and distilling) with 10 ml of  $\text{H}_2\text{SO}_4$  (1+3) and titrating, while still boiling, with the standard permanganate to appearance of pink color.

## 28

## DETERMINATION

Add 10 ml of  $\text{H}_2\text{SO}_4$  (1+3) to 200 ml of sample in porcelain dish and heat to boiling. Add from buret the standard permanganate until the  $\text{H}_2\text{O}$  is distinctly red and boil for 10 min., adding more of the standard permanganate from time to time to maintain the red color. Add 10 ml of the standard oxalic acid and titrate back with the standard permanganate to pink color. From total number of ml of permanganate used, subtract number of ml equivalent to 10 ml of the oxalic acid. The result is number of ml of the permanganate required for 200 ml of the  $\text{H}_2\text{O}$ . Correct for sulfides, nitrites, and ferrous salts, if present, by subtracting the number of ml of the standard permanganate absorbed by another 200 ml portion of the sample when treated as above, except to digest at room temp. and for 3 min.

*Method II<sup>6</sup>—Official*

(For water of high chloride content.)

29

## REAGENTS

*Sodium hydroxide soln.*—Dissolve 50 g of NaOH in H<sub>2</sub>O, cool, and make to 100 ml. The other reagents and solns used are described under 27.

30

## DETERMINATION

Introduce 100 ml of sample into 300 ml flask, add 0.5 ml of the NaOH soln and 10 ml of the permanganate, boil for 10 min., allow to cool to 50–60°, and add 5 ml of H<sub>2</sub>SO<sub>4</sub> (1+3) and 10 ml of the standard oxalic acid. As soon as the liquid has become perfectly colorless, and while constantly agitating, cautiously add the standard permanganate from buret dropwise, until liquid acquires faint permanent redness. The permanganate used is the quantity required for decomposition of the organic matter in 100 ml of sample.

If 100 ml of the sample requires more than 4 ml of the permanganate for oxidation of organic matter, make second determination, using more of the permanganate and a correspondingly larger quantity of the NaOH, as undecomposed permanganate remaining after boiling must be at least twice as great as quantity decomposed.

DISSOLVED OXYGEN<sup>7</sup>*Method I.—Official*

(When more than 0.1 mg of nitrite nitrogen per liter is present.)

31

## REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 6.32 g of KMnO<sub>4</sub> in H<sub>2</sub>O and dilute to 1 liter.

(b) *Potassium oxalate soln.*—Dissolve 20 g of K oxalate in H<sub>2</sub>O and dilute to 1 liter.

(c) *Manganous sulfate soln.*—Dissolve 480 g of MnSO<sub>4</sub>·4H<sub>2</sub>O in H<sub>2</sub>O and dilute to 1 liter.

(d) *Alkaline potassium iodide soln.*—Dissolve 500 g of NaOH and 150 g of KI in H<sub>2</sub>O and dilute to 1 liter.

(e) *Sodium thiosulfate soln.*—0.025 N. Dissolve 6.205 g of A.C.S. reagent-grade Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in H<sub>2</sub>O and dilute to 1 liter with freshly boiled and cooled H<sub>2</sub>O. 1 ml = 0.2 mg of O or 0.1400 ml of O at 0° and 760 mm pressure. As this soln is not permanent it should be standardized occasionally against a 0.025 N soln of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>.

32

## COLLECTION OF SAMPLE

Collect sample in narrow-necked glass-stoppered bottle of 250–270 ml capacity by means of apparatus designed to avoid entrainment or absorption of any O from the atmosphere. Note temp.

33

## DETERMINATION

Remove stopper from bottle, and add 0.7 ml of H<sub>2</sub>SO<sub>4</sub> and then 1 ml of the KMnO<sub>4</sub> soln. Introduce these and all other reagents by pipet under surface of liquid. Insert stopper and mix by inverting bottle several times. If a noticeable excess of KMnO<sub>4</sub> is not present after 20 min., again add 1 ml of the permanganate soln; if this is still insufficient, use a stronger permanganate soln. After 20 min. destroy excess of permanganate by adding 1 ml of the K oxalate soln, re-stopper bottle at once, and mix its contents. Add 1 ml of the MnSO<sub>4</sub> soln and 3 ml of the alkaline KI soln. Allow precipitate to settle. Add 1 ml of H<sub>2</sub>SO<sub>4</sub> and mix by shaking. Transfer

200 ml of contents of bottle to flask and titrate with the 0.025 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , using a few ml of starch indicator, VI, 3(e) *toward the end* of the titration. Do not add the starch soln until color has become faint yellow. Titrate until blue color disappears. Report results in mg per liter; if desired, report results also as percentage of saturation.<sup>8</sup>

34

*Method II.—Official*

(When less than 0.1 mg of nitrite nitrogen per liter is present.)

For reagents and collection of sample, see 31 and 32. Remove stopper from bottle and proceed as directed under 33, beginning "Add 1 ml of the  $\text{MnSO}_4$  soln."

LEAD<sup>9</sup>

(When present in small quantities.)

*Method I.—Tentative*

(Coloring matter, iron, lead, copper, and zinc present.)

35

## REAGENTS

(a) *Ammonium acetate soln.*—Dissolve 200 g of  $\text{NH}_4$  acetate in  $\text{H}_2\text{O}$  and dilute to 500 ml. The soln should be practically colorless.

(b) *Dilute ammonium acetate soln.*—Dilute 50 ml of (a) to 500 ml.

(c) *Standard lead soln.*—Add  $\text{H}_2\text{SO}_4$  in slight excess to 10% soln of Pb acetate. Filter off the  $\text{PbSO}_4$  and wash free from acid with  $\text{H}_2\text{O}$ . Dissolve the  $\text{PbSO}_4$  in the  $\text{NH}_4$  acetate soln, (a), dilute to definite volume, and determine the Pb as  $\text{PbCrO}_4$  by precipitating with  $\text{K}_2\text{CrO}_4$  soln (cf. VI, 39). Dilute stock soln so that 1 ml will contain 0.1 mg of Pb.

36

## REMOVAL OF COLOR

Acidify 0.5–2 liters of sample with  $\text{HCl}$  (1+1) and concentrate in porcelain casserole to volume of ca 75 ml by heating slowly over open flame. Add sufficient  $\text{NH}_4\text{Cl}$  (ca 2 g) to hold Mg in soln and assist in separation of sulfides. Add ca 1 ml of  $\text{NH}_4\text{OH}$  in excess and saturate with  $\text{H}_2\text{S}$ . Cover dish, allow to stand ca 2 hours, add more of the  $\text{NH}_4\text{OH}$  and  $\text{H}_2\text{S}$ , boil a few minutes, let precipitate settle, filter, and wash precipitate once with hot  $\text{H}_2\text{O}$ . (The precipitate will contain all the Fe, Pb, Cu, and Zn, and the coloring matter will be in filtrate.) Place filter and precipitate in small porcelain casserole, add 30 ml of  $\text{HNO}_3$  (1+3) and boil. Filter, wash free from acid, and cool filtrate (soln A).

37

## DETERMINATION

Add to soln A, 36, 5 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of  $\text{SO}_3$  are given off. Cool, wash down sides with a little  $\text{H}_2\text{O}$ , and repeat evaporation and heating. Transfer to beaker with aid of  $\text{H}_2\text{O}$ , add an equal volume of 95% alcohol, and let stand overnight. Filter off the  $\text{PbSO}_4$  and wash with dilute alcohol, 50% by volume, until free from Fe. Collect filtrate, which contains Fe, Cu, and Zn, in 250 ml beaker (soln B). Digest the filter containing the  $\text{PbSO}_4$  in small porcelain casserole with ca 40 ml of the warm  $\text{NH}_4$  acetate soln, filter, and wash once or twice with the warm dilute  $\text{NH}_4$  acetate soln and twice with  $\text{H}_2\text{O}$ . Dilute filtrate to definite volume. To aliquot add freshly prepared  $\text{H}_2\text{S}$  water and a few drops of acetic acid (1+1). Compare color obtained with a set of standards made by treating various quantities of the standard Pb soln with  $\text{H}_2\text{S}$  water.

38

*Method II.—Tentative*

(Coloring matter present; iron present to extent of 1 mg or less in quantity of sample taken for analysis; copper and zinc absent.)

Remove coloring matter as directed under 36. Add to soln A 5 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of  $\text{SO}_3$  are given off. Cool, wash down sides with a little  $\text{H}_2\text{O}$ , and repeat evaporation and heating. Transfer to beaker with aid of  $\text{H}_2\text{O}$ , add 25–40 ml of the  $\text{NH}_4$  acetate soln, 35, heat to boiling, and precipitate the Fe with  $\text{NH}_4\text{OH}$ . Filter and wash with the dilute  $\text{NH}_4$  acetate soln and  $\text{H}_2\text{O}$ . Acidify filtrate slightly with acetic acid (1+1) and determine Pb in filtrate colorimetrically by addition of freshly prepared  $\text{H}_2\text{S}$  water as directed under 37.

39

*Method III.—Tentative*

(Coloring matter, iron, copper, and zinc absent.)

Add 5 ml of  $\text{H}_2\text{SO}_4$  (1+1) to 0.5–2 liters of sample, evaporate nearly to dryness, and heat until copious fumes of  $\text{SO}_3$  are given off. Transfer to beaker with aid of  $\text{H}_2\text{O}$ , add 25–40 ml of the  $\text{NH}_4$  acetate soln, 35(a), and determine Pb colorimetrically by addition of  $\text{H}_2\text{S}$  water as directed under 37.

40

*Method IV.—Tentative*

(Coloring matter absent; iron, lead, copper, and zinc present.)

Add 5 ml of  $\text{H}_2\text{SO}_4$  (1+1) to 0.5–2 liters of sample, evaporate nearly to dryness, and heat until copious fumes of  $\text{SO}_3$  are given off. Filter off the  $\text{PbSO}_4$  and proceed as directed under 37.

## COPPER—TENTATIVE

(When present in small quantities.)

41

## REAGENTS

(a) *Ammonium nitrate soln.*—Dissolve 10 g of  $\text{NH}_4\text{NO}_3$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(b) *Potassium ferrocyanide soln.*—Dissolve 3.5 g of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml. This soln should be freshly prepared.

(c) *Standard copper soln.*—Dissolve ca 20 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , add 1 ml of  $\text{H}_2\text{SO}_4$ , and dilute to 500 ml. Determine the Cu in 50 ml of this soln as  $\text{CuO}$  by precipitation with  $\text{KOH}$  soln. Dilute stock soln so that 1 ml contains 0.1 mg of Cu.

42

## DETERMINATION

Boil the moderately acid filtrate (soln B), 37, which contains Fe, Cu, and Zn, to remove alcohol; adjust soln to 200 ml volume; and add 1 g of  $\text{NH}_4\text{Cl}$ . Heat to boiling, saturate with  $\text{H}_2\text{S}$  gas, and boil to remove precipitated S. Cover beaker, let stand ca 2 hours or until supernatant liquid becomes clear, filter, and wash the  $\text{CuS}$  without intermission with  $\text{H}_2\text{O}$  containing  $\text{H}_2\text{S}$ . Collect the filtrate, soln C, in porcelain casserole. Dissolve precipitate of  $\text{CuS}$  in hot  $\text{HNO}_3$  (1+3). Cool, add a few drops of phenolphthalein indicator, and make soln slightly alkaline with  $\text{NH}_4\text{OH}$  added carefully from a dropping bottle. Add 10 ml of the  $\text{NH}_4\text{NO}_3$  soln, adjust volume to 100 ml, and boil gently until test with red litmus paper shows soln to be neutral. Filter soln to remove any Fe that may be present and adjust filtrate to volume of 100 ml. To an aliquot add 3 drops of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. Compare color



obtained with standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg of Cu. Prepare these standards by measuring corresponding quantities of the standard Cu soln; adding phenolphthalein indicator, a slight excess of  $\text{NH}_4\text{OH}$ , and 10 ml of the  $\text{NH}_4\text{NO}_3$ ; boiling the soln until neutral to red litmus, cooling, and adding 3 drops of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. Make the colorimetric comparison in 100 ml Nessler jars.

## ZINC—TENTATIVE

(When present in small quantities.)

43

## REAGENTS

(a) *Citric acid soln.*—Dissolve 50 g of citric acid crystals ( $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ ) in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(b) *Ammonium thiocyanate soln.*—Dissolve 20 g of  $(\text{NH}_4)\text{SCN}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(c) *Standard zinc soln.*—Dissolve pure Zn in HCl and dilute so that 1 ml contains 0.1 mg of Zn.

(d) *Potassium ferrocyanide soln.*—Dissolve 3.5 g of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

44

## DETERMINATION

Boil the acid filtrate (soln C), 42, from the  $\text{CuS}$  precipitation to remove  $\text{H}_2\text{S}$ , cool, neutralize with  $\text{NH}_4\text{OH}$ , and add 10 ml of the citric acid soln. Heat to boiling, and if no Ca citrate separates add small quantities of powdered  $\text{CaCO}_3$  until precipitate of ca 1 g of Ca citrate is formed. Pass  $\text{H}_2\text{S}$  thru soln until it is cool. Let stand several hours, part of time on steam bath, until supernatant liquid is clear.

Filter, wash with the  $(\text{NH}_4)\text{SCN}$  soln and dissolve precipitate on filter with hot HCl (1+9). If filtrate is reddish in color, reprecipitate the Zn as before. Dispel turbidity of filtrate due to colloidal S by boiling. When filtrate is clear and colorless, dilute an aliquot to 45 ml in 50 ml Nessler jar. Add 5 ml of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln, mix quickly, and compare turbidity with standard Zn solns by viewing longitudinally the jars held over sheet of fine print. Prepare standards by mixing definite volumes of the standard Zn soln, 3 ml of HCl,  $\text{H}_2\text{O}$  to make 45 ml, and 5 ml of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. The unknown soln should contain a volume of acid equivalent to that in standards. Do not use Zn borosilicate glassware in this determination.

## MINERAL WATER

45

## SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at  $20/20^\circ$  by means of pycnometer (XIV, 3).

46

## SOLIDS IN SOLUTION—OFFICIAL.—See 7.

47

## IGNITED RESIDUE—OFFICIAL.—See 8.

48

## NITROGEN IN FORM OF FREE AND ALBUMINOID AMMONIA—OFFICIAL.—See 11 or 13.

49

## NITROGEN IN THE FORM OF NITRITE—OFFICIAL.—See 15.

50

## NITROGEN IN THE FORM OF NITRATE—OFFICIAL.—See 17 or 19.

51

## CHLORIDE—OFFICIAL.—See 21.

HYDROGEN SULFIDE<sup>10</sup>—OFFICIAL

52

## REAGENTS

(a) *Iodine soln.*—0.02 N. Dissolve 10 g of KI (free from iodic acid) in liter flask, using as little  $\text{H}_2\text{O}$  as possible. Add 2.54 g of resublimed I and dissolve by shaking.

Dilute to mark with  $\text{H}_2\text{O}$ . Standardize against a  $\text{Na}_2\text{S}_2\text{O}_3$  soln that has been recently standardized against a  $\text{K}_2\text{Cr}_2\text{O}_7$  soln.

(b) *Iodine soln.*—0.01 *N*. Mix equal volumes of (a) and boiled  $\text{H}_2\text{O}$ . Standardize against a  $\text{Na}_2\text{S}_2\text{O}_3$  soln as directed under (a).

53

## DETERMINATION

Transfer a quantity of sample to graduated vessel by means of siphon and add a few drops of phenolphthalein indicator. If alkaline, add  $\text{HCl}$  until pink color of indicator disappears. Add starch indicator, VI, 3(e), and with careful stirring titrate with the I soln, (a) or (b), until permanent blue color appears. Correct for quantity of I soln needed to give an equally blue color. From corrected quantity of I soln used, calculate approximate quantity of  $\text{H}_2\text{S}$  present. For accurate determinations siphon 100–500 ml of sample, according to quantity of  $\text{H}_2\text{S}$  present, into graduated vessel, keeping outlet of siphon below the liquid. Add immediately sufficient quantity of  $\text{HCl}$ , calculated from the approximate determination, to make neutral to phenolphthalein indicator. Mix carefully with a bent glass rod, and without delay add ca 0.5 ml less I reagent (a) or (b) than is needed to combine with the  $\text{H}_2\text{S}$  present.

Add 5 ml of starch indicator, VI, 3(e), and finish titration with the I soln dropwise with stirring until a blue color remains permanently. Correct for quantity of I soln needed to give an equally blue color when same quantity of starch soln is added to an approximately equal volume of boiled  $\text{H}_2\text{O}$ . If possible, make several determinations and take an average. Standardize reagents (a) and (b) frequently.

54

## FREE CARBON DIOXIDE—TENTATIVE

If sample reacts acid to phenolphthalein and alkaline to methyl orange, titrate 100 ml with 0.05 *N*  $\text{Na}_2\text{CO}_3$  (free from bicarbonate) until soln is neutral to phenolphthalein. No. of ml used  $\times 1.1 = \text{mg}$  of free  $\text{CO}_2$  in 100 ml. Express results in mg per liter.

55

## CARBONIC AND BICARBONIC ACIDS—OFFICIAL

To 100 ml of sample add a few drops of phenolphthalein, and if a pink color is produced titrate with 0.05 *N*  $\text{HCl}$  or  $\text{H}_2\text{SO}_4$ , adding a drop every 2–3 seconds until color disappears. Multiply buret reading by factor 3 to obtain mg of  $\text{CO}_3$  ion in 100 ml. To the colorless soln from this titration, or to the original soln if no color is produced with phenolphthalein, add 1 or 2 drops of methyl orange, continue titration without refilling buret, and note total reading. If  $\text{CO}_3$  is absent, multiply total buret reading by factor 3.05 to obtain the value of  $\text{HCO}_3$  ion in mg per 100 ml. If  $\text{CO}_3$  is present, multiply reading with phenolphthalein by 2 and subtract from total reading of buret. Multiply difference by 3.05 to obtain the  $\text{HCO}_3$  ion in mg per 100 ml. Express results as mg per liter.

56

## SILICA—OFFICIAL

Make preliminary examination, using 100–250 ml of sample, to determine the approximate quantity of Ca and Mg present, in order to ascertain quantity of sample to be evaporated for final analysis.

Evaporate quantity sufficient to yield 0.1–0.6 g of  $\text{CaO}$  or 0.1–1 g of  $\text{Mg}_2\text{P}_2\text{O}_7$  (usually 1–5 liters). Acidify the  $\text{H}_2\text{O}$  with  $\text{HCl}$  and evaporate on steam bath to dryness in Pt dish. Continue drying ca 1 hour. Thoroughly moisten residue with 5–10 ml of  $\text{HCl}$ . Allow to stand 10–15 min. and add sufficient  $\text{H}_2\text{O}$  to bring soluble salts into soln. Heat on steam bath until soln of salts is effected. Filter to remove most of the  $\text{SiO}_2$  and wash thoroughly with hot  $\text{H}_2\text{O}$ . Evaporate filtrate to dryness and treat

residue with 5 ml of HCl and sufficient  $H_2O$  to effect soln of soluble salts, as before. Heat, filter, and wash thoroly with hot  $H_2O$ . Designate the filtrate as soln A. Transfer the 2 residues to Pt crucible, ignite, heat over blast lamp, and weigh. Moisten contents of crucible with a few drops of  $H_2O$ . Add a few drops of  $H_2SO_4$  and a few ml of HF, evaporate on steam bath under hood. Repeat treatment if all  $SiO_2$  is not volatilized. Dry carefully on hot plate, ignite, heat over blast lamp, and weigh. The difference between the 2 weights is the weight of the  $SiO_2$ . Add weight of residue ( $Fe_2O_3 + Al_2O_3$ ) to that of the total  $Al_2O_3$  and  $Fe_2O_3$  obtained under 57. (If the residue weighs more than 0.5 mg,  $BaSO_4$  may be present in the  $H_2O$ . If so, make necessary correction and add to weight of total  $Fe_2O_3$  and  $Al_2O_3$  under 57.)

57

## IRON AND ALUMINUM—OFFICIAL

Concentrate soln A, 56, to 200 ml; while still hot, add  $NH_4OH$  slowly, with constant stirring, until alkaline to methyl orange. Boil, filter, and wash 3 times with hot  $H_2O$ . Dissolve precipitate in hot HCl (1+1). Dilute to ca 25 ml, boil, and again precipitate with  $NH_4OH$ . Filter, wash thoroly with hot  $H_2O$ , dry, ignite, and weigh as  $Al_2O_3$  and  $Fe_2O_3$ . (In presence of  $H_3PO_4$ , the weight of this residue must be corrected for the  $P_2O_5$  equivalent to the  $H_3PO_4$  found under 70, allowance being made for the difference in the volumes of the  $H_2O$  used for these determinations.) Designate the filtrate as soln B.

## IRON

58

*Colorimetric Method—Official*

(Quantity of iron less than 1 mg. Not applicable in presence of phosphates.)

Fuse in Pt crucible the ignited precipitate of  $Fe_2O_3$  and  $Al_2O_3$  with fused  $KHSO_4$ , dissolve in  $H_2O$ , and precipitate the Fe and Al with  $NH_4OH$ . Dissolve precipitate on the filter paper in HCl and  $HNO_3$ , dilute soln, add 5%  $(NH_4)SCN$  soln, and compare color developed with that of calibrated color disks or standards containing known quantities of Fe.

59

*Volumetric Method—Official*

Fuse in Pt crucible the residue of  $Fe_2O_3$  and  $Al_2O_3$  with fused  $KHSO_4$ . This fusion takes but a few minutes and must not be continued beyond time actually needed. When fusion is completed, set crucible aside and allow to cool. Add  $H_2SO_4$  (1+4) and heat crucible until the fused mass is dissolved. Evaporate on steam bath as far as possible; then heat gradually until copious fumes of  $SO_3$  are given off. Dissolve in  $H_2O$  and allow to stand on steam bath. Cool, transfer to Erlenmeyer flask, and make up to such a volume that the soln does not contain more than 2.5% of free  $H_2SO_4$ . Pass  $H_2S$  thru the soln to reduce the Fe and precipitate any Pt contaminating residue from the fusion. (Zn may be used instead of  $H_2S$  for reducing the Fe.) Filter, wash, and again pass  $H_2S$  thru soln so that all the Fe will be reduced. Expel the  $H_2S$  by boiling, at same time passing current of  $CO_2$  thru soln. Test escaping gas with Pb acetate paper to ascertain complete removal of  $H_2S$ . Discontinue boiling and let flask cool without discontinuing the current of  $CO_2$ . Titrate the reduced Fe with standard permanganate soln, 1 ml = 1 mg of Fe, and calculate as Fe.

60

## ALUMINUM—OFFICIAL

To obtain the weight of  $Al_2O_3$ , in absence of phosphates, subtract from the weight of  $Fe_2O_3$  and  $Al_2O_3$ , 57, the Fe, 58 or 59, calculated to  $Fe_2O_3$ . Calculate to Al.

61

## CALCIUM—OFFICIAL

Concentrate soln B, 57, to 150–200 ml, and to this soln, containing an equivalent of not more than 0.6 g of CaO, or 1 g of  $Mg_2P_2O_7$ , add 1–2 g of oxalic acid and sufficient HCl (1+1) to clear the soln. Heat to boiling and neutralize with  $NH_4OH$ , stirring constantly. Add the  $NH_4OH$  in slight excess and allow to stand 3 hours in warm place. Filter off supernatant liquid and wash precipitate once or twice by decantation with 1%  $NH_4$  oxalate soln. Dissolve precipitate in HCl (1+1), dilute to 100–200 ml, add a little more oxalic acid, and precipitate as above. After allowing precipitate to stand 3 hours, filter, wash with the 1%  $NH_4$  oxalate soln, dry, ignite, heat over blast lamp, and weigh as CaO and SrO. Subtract from this weight, the weight of SrO equivalent to the Sr, 62. The difference is the weight of CaO. Calculate to Ca. Designate combined filtrates and washings as soln C.

As a check on the CaO, evaporate to dryness the filtrate from the  $Sr(NO_3)_2$  under 62, beginning "Filter, and wash with ether-alcohol mixture, etc."; dissolve the  $Ca(NO_3)_2$  in  $H_2O$ , precipitate as oxalate, filter, wash, ignite, and weigh as CaO.  $CaO \times 0.7147 = Ca$ .

62

STRONTIUM<sup>11</sup>—TENTATIVE

Dissolve the oxides, 61, in  $HNO_3$  (1+1) and test with spectroscope for Sr. If Sr is present, transfer the  $HNO_3$  soln to a small Erlenmeyer flask. Evaporate nearly to dryness over low flame and heat in air bath at 150–160° for 1–2 hours after the  $H_2O$  is evaporated. Break up the dried material with stirring rod and add 10–15 ml of a mixture of equal parts of absolute alcohol and ether to dissolve the  $Ca(NO_3)_2$ . Cork flask and allow to stand with frequent shaking for 2 hours or longer. Decant the soln thru 5.5 cm filter, reserving filtrate. Wash residue several times by decantation with small portions of ether-alcohol soln. Dry residue and filter paper and wash filter paper repeatedly with small portions of hot  $H_2O$ , collecting filtrate in the flask containing the main portion of the  $Sr(NO_3)_2$  residue. Add 1 or 2 drops of  $HNO_3$  (1+1), evaporate, dry, pulverize, and treat with 10–15 ml of the ether-alcohol mixture. Cork flask and let stand ca 12 hours with occasional shaking. Filter, and wash with ether-alcohol mixture until a few drops of filtrate evaporated on watch-glass leave practically no residue. Dry paper and precipitate. Dissolve the  $Sr(NO_3)_2$  in a few ml of hot  $H_2O$ . Add a few drops of  $H_2SO_4$  and then a volume of alcohol equal to the volume of the soln and allow to stand 12 hours. Filter, ignite, weigh as  $SrSO_4$ , and calculate to Sr. Test spectroscopically for Ca and Ba. If these elements are present, determine quantity and make necessary correction.

63

## MAGNESIUM—OFFICIAL

Concentrate soln C, 61, to 200 ml, acidify with HCl (1+1), and add 2–3 g of  $(NH_4)_2HPO_4$  and sufficient HCl (1+1) to produce a clear soln when all the  $(NH_4)_2HPO_4$  is dissolved. When cold, make slightly alkaline with  $NH_4OH$ , stirring constantly. Add 2 ml excess of  $NH_4OH$  and allow to stand ca 12 hours. Filter off supernatant liquid and wash 4 times by decantation with a soln of  $NH_4OH$  (1+10). Dissolve precipitate in HCl (1+1), dilute to ca 150 ml, add a little  $(NH_4)_2HPO_4$ , and precipitate with  $NH_4OH$  as before. Allow to stand 12 hours, filter, wash free from chlorides with  $NH_4OH$  (1+10), place in porcelain crucible, ignite, heat over blast lamp, and weigh as  $Mg_2P_2O_7$ . Calculate to Mg.  $Mg_2P_2O_7 \times 0.21847 = Mg$ .

64

## SULFURIC ACID—OFFICIAL

Make a preliminary examination, using 100–250 ml of sample, to determine approximate quantity of sulfates. (The alkali salts present can be approximated by

calculating quantity of Na necessary to combine with the excess of acids—HCl,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{CO}_3$ —over the Ca and Mg.

Take a quantity (usually 1–5 liters) sufficient to yield not more than 1 g of  $\text{BaSO}_4$  and not more than 0.5 g of mixed chlorides. Acidify with HCl (1+1), evaporate to dryness in Pt dish, and remove  $\text{SiO}_2$  by two evaporations as directed under 56, using not more than 2 ml of HCl for the final soln. Combine filtrate and washings from the  $\text{SiO}_2$  determinations and concentrate to ca 150–200 ml. Heat to boiling and precipitate with slight excess of 10%  $\text{BaCl}_2$  soln, added very slowly and with constant stirring. Cover, and allow to stand on steam bath ca 12 hours. Filter, thoroly wash precipitate of  $\text{BaSO}_4$  with hot  $\text{H}_2\text{O}$ , until free of chlorides, dry, ignite over Bunsen burner, and weigh.

If the content of sulfate in sample is unusually large, proceed as far as the concentration of the  $\text{SiO}_2$  filtrates, as directed above. Add 50 ml of HCl, heat to boiling, and precipitate with  $\text{BaCl}_2$  soln as before. Evaporate to dryness, take up in  $\text{H}_2\text{O}$  and a few drops of HCl, digest till precipitate settles, wash by decantation, filter, ignite and weigh. Calculate to  $\text{SO}_4$  ion. Designate the filtrate as soln E.

#### SODIUM, POTASSIUM, AND LITHIUM

##### *Ether Alcohol Method*<sup>12</sup>—Official

65

#### PREPARATION OF MIXED CHLORIDES

Evaporate to dryness soln E, 64, in Pt dish, and ignite residue to faint redness to remove all traces of  $\text{NH}_4$  salts. Dissolve residue in dish in ca 200 ml of  $\text{H}_2\text{O}$  and precipitate with milk of lime or a saturated soln of  $\text{Ba}(\text{OH})_2$ . Boil, allow to stand for 30 min., and filter off the insoluble  $\text{Mg}(\text{OH})_2$  and undissolved lime. Thoroly wash precipitate with hot  $\text{H}_2\text{O}$  and combine filtrate and washings. If the precipitate of Mg is large, dissolve it in a small quantity of HCl, evaporate to dryness, take up with  $\text{H}_2\text{O}$ , and precipitate as before. Concentrate the two filtrates and washings to 200–250 ml. Add  $\text{NH}_4\text{OH}$  and sufficient solid  $\text{NH}_4$  carbonate to precipitate the Ca and Ba. Allow to stand on steam bath for 1–2 hours. Filter off supernatant liquid, dissolve precipitate in HCl, reprecipitate as above, and wash thoroly with hot  $\text{H}_2\text{O}$ . Evaporate combined filtrates and washings to dryness and drive off the  $\text{NH}_4$  salts by gentle heat. Treat residue with  $\text{H}_2\text{O}$ , pass thru small filter, using as little wash  $\text{H}_2\text{O}$  as possible, evaporate to small volume, and again precipitate with 1 or 2 drops of  $\text{NH}_4\text{OH}$  and 2 or 3 drops of saturated solns of  $\text{NH}_4$  carbonate and  $\text{NH}_4$  oxalate. If any precipitate appears, filter and repeat process. Evaporate filtrate to dryness and drive off all  $\text{NH}_4$  salts by heating to faint redness in Pt dish. Treat residue with a little  $\text{H}_2\text{O}$ , filter into small Pt dish, add a few drops of HCl (1+1), and evaporate to dryness. Dry in oven, heat to faint redness, cool in desiccator, and weigh the combined chlorides of K, Na, and Li. Repeat heating to constant weight ( $x$ ). Dissolve mixed chlorides in hot  $\text{H}_2\text{O}$ , filter, and wash. Return filter paper and residue to dish, dry, ignite, and weigh ( $y$ ). The difference between ( $x$ ) and ( $y$ ) is the weight of the mixed chlorides.

66

#### DETERMINATION

Dissolve the mixed chlorides, 65, in a minimum quantity of cold  $\text{H}_2\text{O}$  (ca 1.5 ml will be more than sufficient for 0.5 g of the salts), introducing soln into tall 200 ml beaker. Add 1 drop of HCl, and then add gradually 20 ml of absolute alcohol, dropping the alcohol into center of beaker (not on sides) while rotating the soln. (The NaCl and KCl should be precipitated in a perfectly uniform granular condition.)

In a similar manner, add 60 ml of ether (sp. gr. 0.716–0.717 at 25°) and allow mixture to stand ca 5 min. or until precipitate is well agglomerated and supernatant liquid almost clear, rotating mixture occasionally during this period. Filter thru weighed Gooch crucible into Erlenmeyer flask by means of suction, using bell jar arrangement, washing beaker thoroly with mixture of 1 part alcohol and 5 parts ether, and collecting all precipitate on the Gooch with aid of policeman. After thoroly washing precipitate on Gooch, set latter aside and rinse funnel with alcohol-ether mixture to wash any adhering Li soln into the flask containing filtrate. Evaporate filtrate to dryness on steam bath, using air blast. Treat residue with 10 ml of absolute alcohol, warming if necessary, so that practically all residue dissolves. If a slight film remains on bottom and sides of flask, remove with policeman. Then, while rotating soln in flask, add 50 ml of ether (sp. gr. 0.716–0.717 at 25°), followed by 1 drop of HCl. Allow to stand for 30 min., rotating soln at frequent intervals. When the fine precipitate has agglomerated (only very small quantity is usually precipitated), filter into tall beaker by means of suction thru the Gooch crucible containing first precipitate. Wash combined precipitates with the ether-alcohol mixture, taking same precautions as in first precipitation. Dry Gooch and its contents in oven, ignite gently, cool, and weigh to obtain combined weight of NaCl and KCl. Reserve crucible and contents for determination of K.

Evaporate on steam bath the ether-alcohol filtrate and washings containing the Li. Dissolve residue in a little H<sub>2</sub>O, add slight excess of H<sub>2</sub>SO<sub>4</sub> (1+1), and transfer to weighed porcelain or Pt dish. Evaporate as far as possible on steam bath and then gently ignite residue over flame. (By placing dish on a triangle over asbestos gauze and using low flame, the soln can be evaporated without spattering.) Finally ignite carefully over full flame, cool, and weigh. If charring has occurred, repeat ignition with H<sub>2</sub>SO<sub>4</sub>. Calculate to Li, using the factor 0.1263.

Remove the KCl and NaCl from the Gooch crucible by washing with 25–50 ml of hot H<sub>2</sub>O, using suction, and collecting filtrate in porcelain dish. Add sufficient Pt soln, II, 40(b), to convert the KCl and NaCl to K<sub>2</sub>PtCl<sub>6</sub> and Na<sub>2</sub>PtCl<sub>6</sub> and evaporate to dryness. Treat residue with 80% alcohol by volume, filter, and wash until the excess of PtCl<sub>4</sub> and Na<sub>2</sub>PtCl<sub>6</sub> has been removed. Dry filter and precipitate, dissolve residue in hot H<sub>2</sub>O, and transfer to weighed Pt dish. Evaporate on steam bath, dry for 30 min. in oven at 100°, cool, and weigh as K<sub>2</sub>PtCl<sub>6</sub>. Calculate to KCl, using factor 0.3067, and to K, using factor 0.1609.

Determine weight of NaCl by subtracting weight of KCl from weight of combined KCl and NaCl. Calculate to Na, using factor 0.3934.

#### BARIUM

(It is not necessary to look for Ba if sulfate is present in an appreciable quantity unless the H<sub>2</sub>O contains a large quantity of bicarbonate or chloride, which may hold in soln a small quantity of both sulfate and Ba.)

#### Gravimetric Method<sup>13</sup>—Official

#### 67

#### REAGENTS

(a) *Ammonium dichromate soln.*—Dissolve 100 g of the salt free from SO<sub>4</sub> in H<sub>2</sub>O and dilute to 1 liter.

(b) *Ammonium acetate soln.*—Dissolve 300 g of the salt in H<sub>2</sub>O, neutralize with NH<sub>4</sub>OH, and dilute to 1 liter.

(c) *Dilute ammonium acetate soln.*—Dilute 20 ml of (b) to 1 liter.

Reaction of acetate solns should be alkaline rather than acid.

## 68

## DETERMINATION

Acidify a 1-5 liter portion of sample with HCl and concentrate to ca 200 ml. (If precipitate forms, filter it off and examine for Ba.) Add ca 0.5 g of  $\text{NH}_4\text{Cl}$  and precipitate the Fe and Al with  $\text{NH}_4\text{OH}$ . Boil, filter, and wash. To filtrate add an excess of the  $\text{NH}_4$  acetate soln (10 ml), keeping total volume ca 200 ml. Heat to boiling and add, with stirring, ca 5 ml of the  $\text{NH}_4$  dichromate soln. Allow to settle and cool. Decant clear liquid thru filter and wash precipitate by decantation with the dilute  $\text{NH}_4$  acetate soln until filtrate is no longer perceptibly colored (100 ml of wash soln). Place beaker under funnel, dissolve precipitate on paper with warm  $\text{HNO}_3$  (1+1), using as little as possible, and wash the paper. Add a little more acid to dissolve precipitate in beaker, then  $\text{NH}_4\text{OH}$  until precipitate that forms no longer redissolves. Heat to boiling; add with stirring, 10 ml of the  $\text{NH}_4$  acetate soln and 2 ml of the  $\text{NH}_4$  dichromate soln; allow to cool slowly and wash precipitate by decantation with the dilute  $\text{NH}_4$  acetate soln. Dry the  $\text{BaCrO}_4$ , burn filter separately, ignite moderately to constant weight, and weigh as  $\text{BaCrO}_4$ . Record as Ba, using factor 0.54217.

## 69

*Volumetric Method—Official*

Proceed as directed under 68 thru "wash precipitate by decantation with the dilute  $\text{NH}_4$  acetate soln" (after second precipitation). Then proceed as follows: Dissolve precipitate in ca 10 ml of HCl (1+1) and hot  $\text{H}_2\text{O}$ . Wash filter, dilute soln to ca 400 ml, and add ca 50 ml of a freshly prepared 10% soln of KI. Mix carefully and titrate the liberated I after 3 or 4 min. with 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  soln. 1 ml of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3 = 4.579$  mg of Ba.

## 70

## PHOSPHORIC ACID—OFFICIAL

Treat 500 ml of sample, or larger quantity if necessary, with ca 10 ml of  $\text{HNO}_3$  and evaporate in porcelain dish nearly to dryness to drive off HCl. Treat residue with  $\text{H}_2\text{O}$  and filter if necessary. Add  $\text{NH}_4\text{OH}$  to alkalinity and then just enough  $\text{HNO}_3$  to restore acidity. Add some solid  $\text{NH}_4\text{NO}_3$  and heat in  $\text{H}_2\text{O}$  bath at temp. of 45-50°. Add molybdate soln, II, 10(a), and keep at above temp. for 30 min. If more than trace of the yellow precipitate is present, filter and wash with recently boiled and cooled  $\text{H}_2\text{O}$  until entirely free from nitric and molybdic acids. Transfer precipitate and filter to beaker, add a little  $\text{H}_2\text{O}$ , and beat paper and contents to a pulp. Dissolve the yellow precipitate in small quantity of standard KOH, II, 10(b), add phenolphthalein indicator, II, 10(d), and titrate with the standard acid. From data so obtained calculate the  $\text{PO}_4$  ion to mg per liter.

## 71 PREPARATION OF SAMPLE—MANGANESE, IODINE, BROMINE, ARSENIC, AND BORIC ACID

Evaporate 0.5-2 liters of sample to dryness after addition of small quantities of solid  $\text{Na}_2\text{CO}_3$ . Boil residue thus obtained with  $\text{H}_2\text{O}$ , transfer to filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Use residue remaining on filter for determination of Mn. Dilute the alkaline filtrate to definite volume and use for determination of I, Br, As, and  $\text{H}_3\text{BO}_3$ .

## MANGANESE

*I. Persulfate Method—Official*

## 72

## REAGENTS

- (a) *Silver nitrate soln.*—Dissolve 2 g of  $\text{AgNO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.
- (b) *Standard manganous sulfate soln.*—Dissolve 0.2877 g of pure  $\text{KMnO}_4$  in ca

100 ml of  $\text{H}_2\text{O}$ , acidify soln with  $\text{H}_2\text{SO}_4$  (1+1), and slowly heat to boiling. Add slowly sufficient quantity of 10% oxalic acid soln to discharge the color. Cool, and dilute to 1 liter. 1 ml of this soln = 0.1 mg of Mn.

73

## DETERMINATION

Dissolve insoluble residue, 71, in excess of  $\text{HNO}_3$  (1+1), evaporate to dryness, treat with  $\text{H}_2\text{O}$ , and add ca 1 ml of  $\text{HNO}_3$  and a little of the  $\text{AgNO}_3$  soln. If precipitate of  $\text{AgCl}$  appears, add more of the  $\text{AgNO}_3$  until all the Cl is precipitated. Add excess of ca 10 ml of the  $\text{AgNO}_3$  soln for each mg of Mn present in sample. Filter, add 1 g of  $\text{NH}_4$  persulfate to filtrate, and place beaker or flask containing soln on steam bath until pink color develops (ca 20 min.). Compare color developed with standards similarly prepared by treating solns containing known quantities of the standard  $\text{MnSO}_4$  with the dilute  $\text{HNO}_3$ ,  $\text{AgNO}_3$  soln, and  $\text{NH}_4$  persulfate.

II. Bismuthate Method<sup>14</sup>—Official

74

## REAGENTS

- (a) *Nitric acid*.—(1+4). Free from brown oxide of N by aeration.
- (b) *Dilute sulfuric acid*.—Dilute 25 ml of  $\text{H}_2\text{SO}_4$  to 1 liter with  $\text{H}_2\text{O}$ . Add enough  $\text{KMnO}_4$  soln to color faintly.
- (c) *Standard manganous sulfate soln*.—Prepare as directed under 72(b). A soln of  $\text{KMnO}_4$  may be used in place of the  $\text{MnSO}_4$  soln. To prepare it dissolve 0.2877 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

75

## DETERMINATION

Remove Cl by several evaporations with  $\text{H}_2\text{SO}_4$  (1+1) from a quantity of the sample that contains 1 mg or less of Mn. The residue obtained under 71 may be used in place of a fresh sample by dissolving it in an excess of  $\text{HNO}_3$  (1+4), adding the dilute  $\text{H}_2\text{SO}_4$ , and removing Cl by two or more evaporations. In either case, volatilize the  $\text{H}_2\text{SO}_4$  and ignite residue at low heat (less than  $500^\circ$ ). Dissolve in 40 ml of  $\text{HNO}_3$  (1+3), add ca 0.5 g of the Na bismuthate, and heat until permanganate color disappears. Add a few drops of a 10% soln of  $\text{NH}_4$  bisulfite or saturated Na bisulfite to clear the soln and again boil to expel oxides of N. Remove from source of heat, cool to  $20^\circ$ , again add 0.5 g of the Na bismuthate, and stir. When maximum permanganate color has developed, filter thru alundum or Gooch crucible containing an asbestos mat that has been ignited, treated with a 4% soln of  $\text{KMnO}_4$  and washed with  $\text{H}_2\text{O}$ . Wash precipitate with  $\text{H}_2\text{SO}_4$  (1+9) until washings are colorless. Transfer filtrate to colorimeter tube. Compare color developed with standards similarly prepared by treating solns containing known quantities of the standard  $\text{MnSO}_4$  with the dilute  $\text{HNO}_3$ ,  $\text{NH}_4$  or Na bisulfite soln, and Na bismuthate. The color may also be compared with that of standards prepared from the  $\text{KMnO}_4$  soln by diluting portions of 0.2, 0.4, 0.6 ml, etc., of the permanganate soln with the dilute  $\text{H}_2\text{SO}_4$  to the same volume as the filtrate.

## IODIDE AND BROMIDE—TENTATIVE

(This method is qualitative and approximately quantitative. For accurate quantitative methods for iodides, see 102, 105, 109, and 112.)

76

## REAGENTS

- (a) *Sodium hydroxide soln*.—Dissolve 10 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , cool, and dilute to 100 ml.
- (b) *Sodium nitrite soln*.—Dissolve 2 g of  $\text{NaNO}_2$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.



77

## DETERMINATION

Evaporate to dryness an aliquot of alkaline filtrate, 71; add 2–3 ml of  $H_2O$  to dissolve residue and enough 95% alcohol to make percentage of alcohol ca 90. (This precipitates the chlorides.) Heat to boiling, filter, and repeat preceding soln and precipitation once or twice. Add 2 or 3 drops of the NaOH soln to combined alcoholic filtrates and evaporate to dryness. Dissolve this last residue in 2–3 ml of  $H_2O$  and repeat precipitation with alcohol, heating, and filtering. Add a drop of the NaOH to this alcoholic filtrate and evaporate to dryness. Dissolve this residue in a little  $H_2O$ ; acidify with  $H_2SO_4$  (1+5), using 3 or 4 drops in excess; and transfer to small flask. Add 4 drops of the  $NaNO_2$  soln and ca 5 ml of  $CS_2$ . Shake until all the I is extracted and filter off the acid soln from the  $CS_2$ . Wash flask, filter, and contents with cold  $H_2O$  and transfer the  $CS_2$  containing the I in soln to Nessler tube, using ca 5 ml of  $CS_2$ . In washing filter, make contents of tube up to definite volume, usually 12–15 ml, and compare the color with that of other tubes containing known quantities of I dissolved in  $CS_2$ . Prepare these standard tubes by treating measured quantities of a soln of known KI content as described above, beginning "acidify with  $H_2SO_4$  (1+5)."

Transfer separately to small flasks the acid soln of the sample and the standards from which the I has been removed. To standards add definite measured quantities of a bromide soln of known strength, and to each of flasks containing sample and standards add 5 ml of  $CS_2$ . Add saturated and freshly prepared Cl water, 1 ml at a time, shaking after each addition until all the Br is set free. Avoid a large excess of the Cl, as a bromo-chloride may form and spoil the color reaction. Filter off the  $H_2O$  soln from the  $CS_2$  thru moistened filter, wash contents of filter 2 or 3 times with  $H_2O$ , and then transfer to Nessler tube by means of ca 1 ml of  $CS_2$ . Repeat this extraction of filtrate twice, using 3 ml of  $CS_2$  each time. The combined  $CS_2$  extracts usually amount to 11.5–12 ml. Add enough  $CS_2$  to tubes to bring them to definite volume, usually 12–15 ml, and compare sample with standards. If, when using this method near its upper limit, the quantities of  $CS_2$  recommended do not extract all the Br, make one or two extra extractions with  $CS_2$ ; transfer extracts to another tube; and compare color with some of lower standards. Add readings thus obtained to the others.

Results closely approximating the true values for I and Br can be obtained in a shorter time on most samples by omitting the extractions with alcohol and comparing the color of the  $CS_2$  solns directly in the extraction flasks.

## ARSENIC—OFFICIAL

78

REAGENTS AND APPARATUS.—See XXIX, 1 and 2.

79

## DETERMINATION

Take a portion of alkaline filtrate, 71, that contains not more than 0.03 mg of  $As_2O_3$ . If quantity taken is greater than 10 ml, evaporate soln to ca that volume on steam bath. Transfer soln into the generator of the apparatus described under XXIX, with the aid of ca 10 ml of  $H_2O$ , add 20 ml of  $H_2SO_4$  (1+2), and proceed as directed under XXIX, 5, beginning "add 5 ml of the KI reagent."

80

## BORIC ACID—OFFICIAL

(Glassware containing boron must not be used in this determination.)

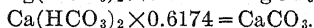
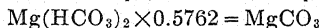
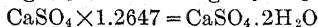
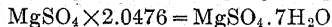
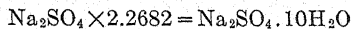
*Qualitative test.*—Evaporate to dryness a part of the alkaline filtrate, 71, treat with 1–2 ml of  $H_2O$ , and slightly acidify with HCl (1+1). Add ca 25 ml of 95% alcohol, boil, filter, and repeat extraction of residue. Make filtrate slightly alkaline with NaOH soln and evaporate to dryness. Add a little  $H_2O$ , slightly acidify with

the dilute HCl, and place a strip of turmeric paper in the liquid. Evaporate to dryness on steam bath and continue heating until turmeric paper is dry. If  $\text{H}_3\text{BO}_3$  is present, the turmeric paper takes on cherry-red color. As a confirmatory test, apply a drop of  $\text{NH}_4\text{OH}$  (1+1) to the reddened paper. A dark olive color will be due to boric acid (cf. XXXII, 16).

*Quantitative method.*—Use the Gooch method.<sup>15</sup>

#### 81 METHOD OF REPORTING RESULTS IN WATERS AND BRINE<sup>16</sup>—TENTATIVE

Report radicals and anhydrous salts in terms of mg per liter or, in the case of highly concentrated waters, in terms of g per liter. For the benefit of physicians, in the case of medicinal waters, report also the salts in terms of grains per quart, using factor 0.014600 to convert mg per liter to grains per quart. In reporting salts in terms of grains per quart, convert the salts that have water of crystallization to the hydrated form as expressed in U. S. Pharmacopoeia and in National Formulary, and convert the  $\text{Mg}(\text{HCO}_3)_2$  to  $\text{MgCO}_3$  and  $\text{Ca}(\text{HCO}_3)_2$  to  $\text{CaCO}_3$ . Use following factors in these calculations:



When a complete analysis is made report error of analysis and state how it is distributed. Report only significant figures.

Report Fe and Al together when present in unimportant quantities and in calculations consider them as Fe. When Fe and Al are present in larger quantities, make the separation and report each separately.

In calculating the hypothetical combinations of acid and basic ions, join  $\text{NO}_2$ ,  $\text{NO}_3$ ,  $\text{BO}_2$ , and  $\text{AsO}_4$  to Na; I and Br to K; and  $\text{PO}_4$  to Ca. Assign the residual basic ions in the following order:  $\text{NH}_4$ , Li, K, Na, Mg, Ca, Sr, Mn, Fe, and Al, to the residual acid ions in the following order: Cl,  $\text{SO}_4$ ,  $\text{CO}_3$ , and  $\text{HCO}_3$ . In case  $\text{HCO}_3$  is not present in sufficient quantity to join with all the Ca, the residual Ca is joined to  $\text{SiO}_2$  to form  $\text{CaSiO}_3$ , and Mn, Fe, and Al are calculated to the oxides  $\text{Mn}_3\text{O}_4$ ,  $\text{Fe}_2\text{O}_3$ , and  $\text{Al}_2\text{O}_3$ , respectively.

Use equivalent combining weights or their reciprocals in uniting the radicals, and when necessary for the purpose of comparison, in reducing salts to radicals and reuniting the radicals in the order specified above.

The equivalent combining weight of a radical is obtained by dividing its weight by its valence. The equivalent combining weight of a salt is obtained by dividing its molecular weight by the product of the valency of the basic element and the number of atoms of the basic element in the salt.

The procedure in calculating the hypothetical combinations by the use of the equivalent combining weights and their reciprocals is as follows:

Multiply the weights obtained, expressed in mg per liter, or, in the case of highly concentrated waters, in g per liter, for each radical to be combined, by the corresponding reciprocal of the equivalent combining weights. If the Na and K are to be determined by calculation, as is frequently the case, subtract the sum of the values obtained (reacting values) for the basic radicals from the sum of the reacting values for the acid radicals. The difference represents the reacting value of the undetermined Na and K. When all the constituents in the  $\text{H}_2\text{O}$  have been determined the sums of the reacting values of the acid and of the basic radicals should be very nearly the same. In this case, if the difference is reasonable and well within the limit of accuracy of the methods used, it may be distributed equally among all the radicals

determined, or among those that the analyst believes to be less accurate than the others. If the difference is unreasonably great, repeat the analysis in whole or in part. The sums of the reacting values of the acid and basic radicals must be equal before proceeding with the calculation. Obtain the reacting values of the salts by subtracting in succession the reacting values of the radicals in the specified order. To convert these values to mg per liter of the respective salts multiply each of them by the equivalent combining weight of the respective salt.

**82**      *Equivalent Combining Weights and Their Reciprocals Based on  
International Atomic Weights, 1939*

NEGATIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS	POSITIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS
NO <sub>3</sub>	62.008	0.01613	NH <sub>4</sub>	18.0404	0.05543
BO <sub>2</sub>	42.82	0.02335	Li	6.940	0.14409
AsO <sub>4</sub>	46.30	0.02160	K	39.096	0.02558
I	126.92	0.00788	Na	22.997	0.04348
Br	79.916	0.01251	Mg	12.16	0.08224
PO <sub>4</sub>	31.660	0.03158	Ca	20.04	0.04990
HS	33.0681	0.03024	Sr	43.815	0.02282
S	16.03	0.06238	Ba	68.68	0.01456
SiO <sub>3</sub>	38.03	0.02630	Mn	27.465	0.03641
O	8.0000	0.12500	Fe <sup>11</sup>	27.92	0.03582
Cl	35.457	0.02820	Fe <sup>111</sup>	18.613	0.05372
SO <sub>4</sub>	48.03	0.02082	Al	8.99	0.11123
CO <sub>3</sub>	30.005	0.03333	Cu	31.785	0.03146
HCO <sub>3</sub>	61.018	0.01639			

SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS	SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS
NH <sub>4</sub> Cl	53.4974	0.01869	MgSO <sub>4</sub>	60.19	0.01661
LiCl	42.397	0.02359	MgCO <sub>3</sub>	42.16	0.02372
Li <sub>2</sub> SO <sub>4</sub>	54.970	0.01819	Mg(HCO <sub>3</sub> ) <sub>2</sub>	73.158	0.01366
Li <sub>2</sub> CO <sub>3</sub>	36.945	0.02707	Mg(NO <sub>3</sub> ) <sub>2</sub>	74.168	0.01348
LiHCO <sub>3</sub>	67.958	0.01472	CaCl <sub>2</sub>	55.497	0.01802
KCl	74.553	0.01341	CaSO <sub>4</sub>	68.07	0.01469
K <sub>2</sub> SO <sub>4</sub>	87.126	0.01148	CaCO <sub>3</sub>	50.045	0.01998
K <sub>2</sub> CO <sub>3</sub>	69.101	0.01447	Ca(HCO <sub>3</sub> ) <sub>2</sub>	81.058	0.01234
KHCO <sub>3</sub>	100.114	0.00999	CaSiO <sub>3</sub>	58.07	0.01722
KI	166.016	0.00602	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	51.70	0.01934
KBr	119.012	0.00840	SrSO <sub>4</sub>	91.845	0.01089
NaCl	58.454	0.01711	SrCO <sub>3</sub>	73.82	0.01355
NaBr	102.913	0.00972	Sr(HCO <sub>3</sub> ) <sub>2</sub>	104.833	0.00954
NaI	149.917	0.00667	BaSO <sub>4</sub>	116.71	0.00857
Na <sub>2</sub> SO <sub>4</sub>	71.027	0.01408	Ba(HCO <sub>3</sub> ) <sub>2</sub>	129.698	0.00771
Na <sub>2</sub> CO <sub>3</sub>	53.002	0.01887	MnSO <sub>4</sub>	75.495	0.01324
NaHCO <sub>3</sub>	84.0151	0.01190	MnCO <sub>3</sub>	57.47	0.01740
NaNO <sub>2</sub>	69.005	0.01449	Mn(HCO <sub>3</sub> ) <sub>2</sub>	88.483	0.01130
NaNO <sub>3</sub>	85.005	0.01176	FeSO <sub>4</sub>	75.95	0.01317
NaBO <sub>2</sub>	65.817	0.01519	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	66.643	0.01500
Na <sub>3</sub> AsO <sub>4</sub>	69.300	0.01443	FeCO <sub>3</sub>	57.925	0.01726
NaF	41.997	0.02381	Fe(HCO <sub>3</sub> ) <sub>2</sub>	88.938	0.01124
NaHS	56.0651	0.01784	Fe <sub>2</sub> O <sub>3</sub>	26.613	0.03758
Na <sub>3</sub> PO <sub>4</sub>	54.657	0.01829	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	57.02	0.01754
Na <sub>2</sub> S	39.027	0.02562	Al <sub>2</sub> O <sub>3</sub>	16.99	0.05886
Na <sub>2</sub> SiO <sub>3</sub>	61.027	0.01639			
MgCl <sub>2</sub>	47.617	0.02100			

## INDUSTRIAL WATER

83 SOLIDS IN SOLUTION—OFFICIAL.—*See* 7.84 CHLORIDE—OFFICIAL.—*See* 21.85 CARBONIC AND BICARBONIC ACIDS—OFFICIAL.—*See* 55.86 NITRATES—OFFICIAL.—*See* 17 or 19.

87 SILICA—OFFICIAL

Proceed as directed under 56. Generally one evaporation with HCl for removal of  $\text{SiO}_2$  is sufficient.

88 IRON AND ALUMINUM—OFFICIAL.—*See* 57.

89 CALCIUM—OFFICIAL

If no  $\text{H}_3\text{PO}_4$  is present, concentrate the filtrate from the determination of Fe and precipitate with  $\text{NH}_4\text{OH}$  and oxalic acid as directed under 61. (Usually one precipitation is sufficient.)

90 MAGNESIUM—OFFICIAL.—*See* 63.

91 SULFURIC ACID AND ALKALIES—OFFICIAL

Proceed as directed under 64 and 66. For technical purposes sufficient accuracy is obtained by determining the acids and the bases, except Na and K, and then calculating the excess of acid over basic ions to the Na salt, stating the alkali thus found as Na and K by difference.

92 TEMPORARY HARDNESS—OFFICIAL

The difference between the alkalinity after boiling, 94, and the alkalinity before boiling, 93, is the temporary hardness in p.p.m. of  $\text{CaCO}_3$ .

ALKALINITY<sup>17</sup>93 *I. Before Boiling—Official*

Measure 100 ml of sample into a 250 ml white glass-stoppered bottle; add 2.5 ml of erythrosine (0.1 g of the Na salt in 1 liter of  $\text{H}_2\text{O}$ ), 5 ml of  $\text{CHCl}_3$  (neutral to erythrosine), and 0.02 *N*  $\text{H}_2\text{SO}_4$  in small quantities, shaking bottle vigorously after each addition of acid. The rose color gradually disappears and is finally discharged by 1 or 2 drops of the acid. A white paper held back of the bottle facilitates detection of end point. Multiply the number of ml of 0.02 *N*  $\text{H}_2\text{SO}_4$  used by 10 to obtain number of p.p.m. of alkalinity in terms of  $\text{CaCO}_3$ .

94 *II. After Boiling—Official*

Boil 100 ml of sample in porcelain dish gently for 30 min. Cool, transfer to 100 ml volumetric flask, and fill to mark with recently boiled and cooled  $\text{H}_2\text{O}$ . Filter thru dry paper and determine alkalinity of filtrate as directed under 93, making proper calculation for aliquot used and calculating in terms of  $\text{CaCO}_3$  the p.p.m. of alkalinity after boiling.

95 TOTAL HARDNESS<sup>18</sup>—OFFICIAL

Add sufficient 0.05 *N*  $\text{H}_2\text{SO}_4$  to 200 ml of sample contained in 500 ml Pyrex or similar glass Erlenmeyer flask to neutralize the alkalinity, the quantity required

being calculated from results obtained as directed under 93. Measure 200 ml of  $H_2O$  into similar flask. Treat contents of each flask in following manner: Boil 15 min. to expel free  $CO_2$ , add 25 ml of soda reagent (0.1  $N$ , equal parts of  $NaOH$  and  $Na_2CO_3$ ), boil 10 min., cool, rinse into 200 ml volumetric flasks, and dilute to 200 ml with boiled  $H_2O$ . Filter, rejecting first 50 ml, and titrate 50 ml of each filtrate with the 0.05  $N$   $H_2SO_4$  in the presence of methyl orange or erythrosine indicator. Total hardness (p.p.m. of  $CaCO_3$ ) = 50 times difference between the ml of 0.05  $N$   $H_2SO_4$  used in titrating the aliquot of the blank and the aliquot of the sample.

96

## PERMANENT OR NON-CARBONATE HARDNESS—OFFICIAL

Difference between alkalinity before boiling, 93, and total hardness, 95, = permanent or non-carbonate hardness (p.p.m. of  $CaCO_3$ ).

## IRRIGATING WATER

97

## GENERAL METHODS—OFFICIAL

Determine the solids in soln,  $Cl$ ,  $CO_3$  and  $HCO_3$ ,  $Ca$ ,  $Mg$ , and  $H_2SO_4$  as directed under 7, 21, 55, 61, 63, and 64, respectively. To make the hypothetical combination, calculate  $Ca$  and  $Mg$  to the acid ions in following order:  $HCO_3$ ,  $SO_4$ , and  $Cl$ . Then calculate remaining acid ions, including  $CO_2$ , to corresponding salts of  $Na$ .

BLACK ALKALI<sup>18</sup>—OFFICIAL

98

## REAGENTS

(a) *Sodium carbonate*.—0.02  $N$ . 1 ml = 0.00106 g of  $Na_2CO_3$ .

(b) *Carbon dioxide-free water*.—Boil  $H_2O$  vigorously until ca  $\frac{1}{3}$  of original volume is evaporated, cool, and stopper.

99

## DETERMINATION

Transfer 200 ml of sample to a Pt or Ag dish; add 50–100 ml of the  $Na_2CO_3$  soln, according to quantity of soluble salts of  $Ca$  and  $Mg$  present; and evaporate to dryness. Rub up residue with  $CO_2$ -free  $H_2O$  and transfer to 100 ml volumetric flask. (An ordinary laboratory wash bottle should not be used to transfer the residue, as the  $CO_2$  from breath of operator will vitiate results.) Dilute to mark, shake thoroly, and allow to stand until clear (12–15 hours). Remove 50 ml of the clear supernatant liquid, equivalent to half of original quantity of sample and  $Na_2CO_3$  added, and transfer to 250 ml glass-stoppered flask or a stoppered titration bottle of clear glass, without any tinge of pink. Add 5 ml of  $CHCl_3$ , neutral to erythrosine, and 1 ml of erythrosine indicator (0.1 g of the  $Na$  salt in 1 liter of  $H_2O$ ) and titrate with 0.02  $N$   $H_2SO_4$  until color disappears. Shake soln vigorously after each addition of acid. The milky appearance produced by the  $CHCl_3$  makes reading of end point sharp and certain.

(1) If less  $H_2SO_4$  is required than is equivalent to half of the  $Na_2CO_3$  added, due to some of the  $Na_2CO_3$  reacting with soluble salts of  $Ca$  and  $Mg$ , the soln originally contained no black alkali in excess, but rather an excess of the so-called permanent or non-carbonate hardness. Express hardness in terms of  $CaSO_4$ . Difference between number of ml of the  $H_2SO_4$  required and half the number of ml of the  $Na_2CO_3$  added  $\times$  factor 0.00136 = equivalent of  $CaSO_4$  in 100 ml of sample.

(2) If more  $H_2SO_4$  is required than that equivalent to half of the  $Na_2CO_3$  added, black alkali was originally present in the soln, and the difference in ml  $\times$  factor 0.00106 = black alkali in terms of  $Na_2CO_3$  in 100 ml of sample.

## BRINE

## IODIDE IN THE PRESENCE OF CHLORIDE AND BROMIDE

*Method I.<sup>20</sup>—Tentative*

100

## REAGENTS

(a) *Sodium hydroxide-sodium carbonate soln.*—Dissolve 50 g of a mixture of equal weights of NaOH and Na<sub>2</sub>CO<sub>3</sub> in H<sub>2</sub>O and dilute to 1 liter.

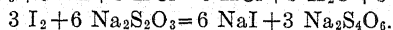
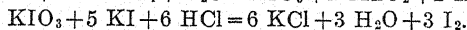
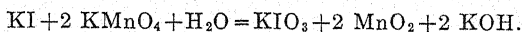
(b) *Sodium hydroxide soln.*—Dissolve 4 g of NaOH in H<sub>2</sub>O and dilute to 100 ml.

(c) *Potassium permanganate soln.*—Dissolve 50 g of KMnO<sub>4</sub> in H<sub>2</sub>O and dilute to 1 liter.

(d) *Sodium thiosulfate soln.*—0.05 N. Dissolve 12.4 g of recrystallized Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · 5H<sub>2</sub>O in H<sub>2</sub>O and dilute to 1 liter.

101

## REACTIONS



102

## DETERMINATION

Take a quantity of the brine that contains not more than 0.1 g of I nor more than 10 g of total salts. Adjust volume to 100–150 ml, and add sufficient Reagent (a) to precipitate the Ca and Mg. Boil, filter off precipitate of Ca and Mg, and wash with hot H<sub>2</sub>O. Introduce filtrate into Erlenmeyer flask, adjust volume to ca 100 ml, neutralize with H<sub>2</sub>SO<sub>4</sub> (1+9), and add 1 ml of the NaOH soln. Heat to boiling; add an excess of ca 0.5 ml of the KMnO<sub>4</sub>; continue heating until precipitate begins to coagulate; and allow to cool. Add sufficient alcohol or H<sub>2</sub>O<sub>2</sub> to bleach the permanganate color and set beaker on steam bath. When precipitate has settled, filter and wash with hot H<sub>2</sub>O. After cooling, add 1–2 g of KI, acidify with HCl, and titrate with the 0.05 N thiosulfate soln. Since one-sixth of the I titrated represents quantity originally present, 1 ml of 0.05 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln = 1.058 mg of I.

*Method II.<sup>21</sup>—Tentative*

103

## REAGENTS

(a) *Potassium iodide soln.*—Dissolve 200 g of KI, free from iodate, in H<sub>2</sub>O and dilute to 1 liter.

(b) *Starch iodide paper.*—Dip strips of filter paper in 25 ml of the starch indicator, VI, 3(e), that has been mixed with 5 ml of a 10% soln of KI.

104

## APPARATUS

(a) *Reaction flask.*—A glass-stoppered flask of 200–400 ml capacity provided with inlet and outlet tubes, the inlet tube having stopcock and reaching nearly to bottom of flask and outlet tube having bulb of ca 25 ml capacity blown near center to lessen danger of the absorbing soln being drawn back into reaction flask.

(b) *Tall absorption bottle.*

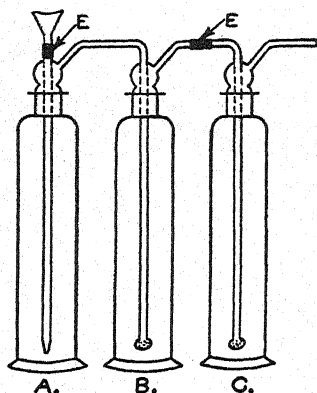
105

## DETERMINATION

Reduce by evaporation a quantity of sample that contains not more than 0.1 g of I to volume of 25 ml, and place in glass-stoppered reaction flask. Add 5 ml of

HCl (1+1), insert stopper, and then add thru inlet tube 50 ml of freshly prepared Cl water. Place end of outlet tube in tall absorption flask in which is placed 35 ml of 10% soln of  $K_2CO_3$  diluted to 150 ml. Heat reaction flask and boil gently until most of the Cl and Br has been distilled into the alkali. Connect inlet tube to a  $CO_2$  generator and complete distillation by simultaneous boiling and bubbling of  $CO_2$  thru sample. Continue for 10 min., testing for presence of Cl and Br by holding a piece of starch iodide paper at the end of outlet tube. Remove source of heat and bubble  $CO_2$  thru apparatus until it is cool. Add 5 ml of the soln of KI and titrate the liberated I with 0.05 N  $Na_2S_2O_3$  soln, 100 (d).

#### BROMIDE IN PRESENCE OF CHLORIDE BUT NOT IODIDE<sup>22</sup>—TENTATIVE



**A. REACTION CYLINDER.  
B & C. ABSORPTION CYLINDERS.  
E. RUBBER CONNECTIONS.**

FIG. 50.—REACTION CYLINDER TO BE USED IN DETERMINATION OF BROMIDE

A by introducing glass beads to depth of ca 1", followed by 15 g of  $CrO_3$  crystals and finally enough glass beads to fill cylinder half full. Add 20 ml of alkaline  $Na_2SO_3$  soln, 106, to cylinder B and 5 ml to cylinder C. Dilute each to ca 200 ml. Connect the three cylinders and draw current of air thru slowly. Wash sample into cylinder A with sufficient  $H_2O$  to make ca 25 ml of soln. Aspirate until contents of this cylinder are in soln and thoroly mixed, close inlet tube with small piece of rubber tubing and clamp, and reduce pressure in apparatus slightly by suction in order to guard against any possible escape of Br at ground-glass stopper. Allow to stand overnight and then aspirate with rather strong current of air (ca 0.5–0.75 liter per min.) for 3 hours, adding four 2 ml portions of 3%  $H_2O_2$  soln to reaction flask at 30 min. intervals. Stop aspiration and evaporate contents of cylinders B and C nearly to dryness. Clean out cylinder A and freshly charge with glass beads and 15 g of  $CrO_3$  crystals. To cylinder B add 10 g of KI crystals dissolved in 200 ml of  $H_2O$  and to C 3 or 4 g in a like quantity of  $H_2O$ . Connect apparatus, draw thru slow current of air, and transfer contents of evaporating dish to cylinder A by means of the small funnel, using 25 ml of  $H_2O$ . Aspirate until all the Br is evolved (ca 1 hour) and titrate the KI soln with standard 0.05 N  $Na_2S_2O_3$  soln, 100(d). 1 ml of  $Na_2S_2O_3$  = 3.996 mg of Br.

106

REAGENT

*Alkaline sodium sulfite soln.*—Dissolve 4 g of  $Na_2SO_3$  and 0.8 g of  $Na_2CO_3$  in  $H_2O$  and dilute to 100 ml.

107

APPARATUS

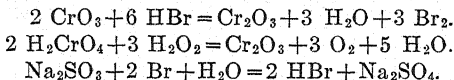
(a) *Reaction cylinder.*

(b) *Two high-form gas washing bottles.*

Join reacting cylinder and the two gas washing bottles as shown in Fig. 50.

108

REACTIONS



109

DETERMINATION

Take a quantity of the brine that contains not more than 10 g of total salts. (Sample should not be too acid.) Evaporate to dryness or nearly so. Charge cylinder

BROMIDE IN PRESENCE OF CHLORIDE AND IODIDE<sup>23</sup>—TENTATIVE

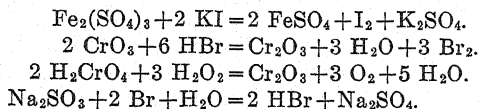
(Collaborative work indicates that the following is the best method that has been published for the determination of Br in the presence of Cl and I, but the results obtained show that only about 95% of the Br present is recovered when 80 mg of Br is contained in the portion of the sample taken for analysis. The method is satisfactory in the absence of I.)

110

REAGENT AND APPARATUS.—See 106 and 107.

111

## REACTIONS



112

## DETERMINATION

Introduce 10 ml of sample into a distillation flask, adjust volume to ca 75 ml, and add 1.5–2.0 g of  $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ . Distil off liberated I with steam, discarding distillate. Transfer residue from distillation flask to a beaker, heat to boiling, add a few drops of methyl orange, and precipitate the Fe with  $\text{NH}_4\text{OH}$ , avoiding an excess of  $\text{NH}_4\text{OH}$ , as a precipitate of  $\text{Ca}(\text{OH})_2$  is bulky and difficult to wash. Filter off the  $\text{Fe}(\text{OH})_3$ , wash with hot  $\text{H}_2\text{O}$ , and evaporate filtrate and washings to dryness or nearly so, taking care that during the evaporation the soln does not become acid from hydrolysis of  $\text{MgCl}_2$ . Proceed as directed under 109, beginning "Charge cylinder A."

SALT<sup>24</sup>

113

## PREPARATION OF SAMPLE—TENTATIVE

If sample is coarser than 20 mesh, grind so that all will pass thru 20-mesh sieve, but avoid undue grinding so that as much as possible will be retained on an 80-mesh sieve. Mix sample by quartering and weigh all needed portions as nearly at the same time as possible.

114

## MOISTURE—TENTATIVE

Place ca 10 g of sample in dry, weighed Erlenmeyer flask of ca 200 ml capacity. Weigh flask and sample. Spread sample evenly over bottom of flask by shaking gently and insert a small funnel in the neck. Heat flask and sample for periods of 1 hour each on triangle over low, open flame of gas stove at ca 250° until two consecutive weighings agree within 5 mg. Shake flask occasionally so that sample will dry evenly. Report loss of weight as moisture.

115

## MATTERS INSOLUBLE IN WATER—TENTATIVE

Place 10 g of sample in a 250 ml beaker, add 200 ml of  $\text{H}_2\text{O}$  at room temp., and let stand 30 min., stirring frequently. Filter thru weighed Gooch crucible with asbestos mat dried at 110°. Transfer residue to a Gooch crucible with aid of a policeman, using total of not more than 50 ml of  $\text{H}_2\text{O}$ . Wash residue with small portions of  $\text{H}_2\text{O}$ , ca 10 portions of 10 ml each, until 10 ml of filtrate shows only faint opalescence upon addition of a few drops of  $\text{AgNO}_3$  soln. Dry crucible and contents to constant weight at 110°. Call increase in weight of Gooch crucible, "matters insoluble in  $\text{H}_2\text{O}$ ," and report results in percentage on a moisture-free basis. If matters insoluble in  $\text{H}_2\text{O}$  exceed 0.1%, determine their nature.



116

MATTERS INSOLUBLE IN ACID<sup>25</sup>—TENTATIVE

Treat 10 g of sample with 200 ml of HCl (1+19), boil 2–3 min., and let stand 30 min., stirring frequently. Filter thru a Gooch crucible with mat, dried at 110°. Express results in percentage.

## 117 PREPARATION OF SOLUTION FOR SULFATE, CALCIUM, AND MAGNESIUM—TENTATIVE

Weigh ca 20 g of sample, transfer to a 400 ml beaker, and dissolve in 200 ml of HCl (1+3). Cover beaker, heat to boiling, and continue boiling gently for 10 min. Filter thru paper filter and wash residue with small quantities of hot H<sub>2</sub>O until filtrate is free from chlorides. Unite filtrate and washings, cool, and make to a volume of 500 ml (soln A).

118

## SULFATE—TENTATIVE

Place 250 ml of soln A, 117, in a 400 ml beaker of resistant glass, heat to boiling, and add a slight excess of hot 10% BaCl<sub>2</sub> soln dropwise while stirring. Concentrate by heating gently and finally evaporate to dryness on steam bath. Facilitate removal of free acid by stirring the partly dried residue. Wash precipitate by decantation with small quantities of hot H<sub>2</sub>O, finally transferring precipitate to a close-grained filter paper with aid of a policeman and stream of hot H<sub>2</sub>O. Test filtrate for presence of Ba. Wash precipitate on filter until filtrate is free from chlorides. Dry and ignite filter containing precipitate over Bunsen flame. Report percentage of SO<sub>4</sub> in sample on moisture-free basis.

119

## CALCIUM—TENTATIVE

Place remainder of soln A in a 400 ml beaker of resistant glass. Add excess of 10% oxalic acid soln (10 ml usually will be sufficient). Add a few drops of methyl orange indicator, neutralize while hot by adding NH<sub>4</sub>OH dropwise, stirring constantly. Add ca 1 ml excess of the NH<sub>4</sub>OH, stir, and let stand in warm place for 3 hours. Decant supernatant liquid thru filter, reserving filtrate for determination of Mg. Test filtrate for Ca with NH<sub>4</sub> oxalate soln. Wash precipitate in a beaker once with 10 ml of a 1% NH<sub>4</sub> oxalate soln, decanting thru filter paper. Combine filtrate and washings. Dissolve precipitate on filter with hot HCl (1+1), using same beaker, dilute to 100 ml, add a little more oxalic acid, and precipitate as before. After allowing to stand 3 hours, filter and wash with the NH<sub>4</sub> oxalate soln as before, reserving filtrate and washings. Transfer precipitate to crucible, dry, ignite, and heat over blast lamp to constant weight. Report as percentage of Ca on moisture-free basis.

120

## MAGNESIUM—TENTATIVE

Combine filtrates and washings from the Ca determination, concentrate if necessary by boiling gently to volume of ca 150 ml, and proceed as directed under 63. Report as percentage of Mg on moisture-free basis.

121

## METHOD OF REPORTING RESULTS ON SALT—TENTATIVE

(In the absence of added drying agents such as MgCO<sub>3</sub>, Ca phosphate, etc.)

Convert the sulfate to CaSO<sub>4</sub> and the unused Ca to CaCl<sub>2</sub>, unless the sulfate in sample exceeds quantity necessary to combine with the Ca, in which case convert the Ca to CaSO<sub>4</sub> and the unused sulfate first to MgSO<sub>4</sub> and the remaining sulfate, if any, to Na<sub>2</sub>SO<sub>4</sub>. Convert unused Mg to MgCl<sub>2</sub>. Add percentages of CaCl<sub>2</sub> and MgCl<sub>2</sub>.

Report on moisture-free basis the percentage of matters insoluble in  $H_2O$ , of  $SO_4$ , of Ca, of Mg, of  $CaSO_4$ , of  $CaCl_2$ , and  $MgCl_2$ . Report also results of qualitative examination of matters insoluble in  $H_2O$ , if the quantity exceeds 0.1 % on moisture-free basis.

## SELECTED REFERENCES

- <sup>1</sup> U. S. Dept. Interior, Bur. Geological Survey, Paper 151, p. 23.
- <sup>2</sup> J. Am. Chem. Soc., 32, 1256 (1910); J. Assoc. Official Agr. Chem., 4, 384 (1921).
- <sup>3</sup> J. Am. Chem. Soc., 31, 922 (1909); 32, 630 (1910); 33, 366, 381 (1911).
- <sup>4</sup> Univ. of Illinois Bull., 7, No. 2, 1909; Water Survey Ser. No. 7, p. 14; J. Assoc. Official Agr. Chem., 4, 92 (1920).
- <sup>5</sup> J. Assoc. Official Agr. Chem., 22, 482 (1939).
- <sup>6</sup> Z. anal. Chem., 8, 344 (1869).
- <sup>7</sup> Ber., 21, 2843 (1888).
- <sup>8</sup> J. Am. Chem. Soc., 33, 362 (1911).
- <sup>9</sup> Rept. Massachusetts State Bd. Health, 1898, p. 582; J. Ind. Eng. Chem., 13, 696 (1921); J. Assoc. Official Agr. Chem., 5, 382 (1922); 6, 307 (1923).
- <sup>10</sup> J. Assoc. Official Agr. Chem., 9, 29 (1926).
- <sup>11</sup> Chem. Ztg., 35, 337 (1911); J. Assoc. Official Agr. Chem., 1, 97, 458 (1915); 2, 113 (1916).
- <sup>12</sup> J. Am. Chem. Soc., 38, 2326 (1916); U. S. Dept. Agr. Bur. Chem. Bull. 153; J. Assoc. Official Agr. Chem., 3, 368 (1920).
- <sup>13</sup> Morse, Exercises in Quantitative Chemistry, p. 417; J. Assoc. Official Agr. Chem., 4, 86 (1920).
- <sup>14</sup> J. Am. Chem. Soc., 34, 1379 (1912); J. Assoc. Official Agr. Chem., 4, 85 (1920).
- <sup>15</sup> Am. Chem. J., 9, 23 (1887).
- <sup>16</sup> J. Assoc. Official Agr. Chem., 5, 385 (1922).
- <sup>17</sup> J. Am. Chem. Soc., 21, 359 (1899).
- <sup>18</sup> Z. angew. Chem., 15, 193 (1902).
- <sup>19</sup> J. Am. Chem. Soc., 28, 77 (1906).
- <sup>20</sup> J. Ind. Eng. Chem., 11, 563 (1919); J. Assoc. Official Agr. Chem., 4, 380 (1921).
- <sup>21</sup> Z. anal. Chem., 39, 81 (1900); J. Assoc. Official Agr. Chem., 5, 381 (1922).
- <sup>22</sup> J. Ind. Eng. Chem., 11, 954 (1919); J. Assoc. Official Agr. Chem., 4, 381 (1921).
- <sup>23</sup> J. Ind. Eng. Chem., 12, 358 (1920); J. Assoc. Official Agr. Chem., 5, 29 (1921).
- <sup>24</sup> J. Assoc. Official Agr. Chem., 5, 384 (1922); 7, 309 (1924).
- <sup>25</sup> Ibid., 5, 385 (1922); 6, 129 (1923).

## XXXVIII. RADIOACTIVITY

1

### QUALITATIVE TEST—OFFICIAL

(Applicable to solids.)

Charge an alpha ray electroscope (preferably of the Lind type) to bring leaf to a suitable position on scale in microscope. Close door and record position of leaf on scale at frequent intervals, until rate of fall of leaf is constant. Calculate rate of fall of leaf in divisions per minute, designating figure obtained as natural leak of instrument for that particular determination.

Place a convenient portion of sample on pan of the electroscope, close door, recharge leaf system, and record rate of fall of leaf in divisions per minute over same range of scale as before, until rate becomes constant, recharging if necessary. A rate of fall in excess of natural leak of instrument shows that sample is radioactive.

### QUANTITATIVE METHODS

#### *Emanation or radon method<sup>1</sup>—Official*

2

#### REAGENTS

(All reagents should be free from radium and radon.)

- (a) *Nitrogen*.—Use tank nitrogen that has been allowed to stand at least 30 days.
- (b) *Standard radium soln*.—Use a stock soln that contains  $1 \times 10^{-9}$  g of radium/100 ml. This soln should be standardized by the National Bureau of Standards. Preserve soln in a sealed Pyrex flask with ca 2%  $\text{BaCl}_2$  and 2%  $\text{HCl}$ .
- (c) *Resublimed  $\text{P}_2\text{O}_5$* .
- (d) *Calcium chloride*.—C.P. fused.
- (e) *Mercury*.—Redistilled.

3

#### APPARATUS

Use an all-glass apparatus (Fig. 51). Provide several units for storing sample which include Nos. 2, 4, 5, 7, 8, 10, and 11.

Nos. 1 and 2 are 300 ml Pyrex flasks. Flask No. 1 has a tube sealed on to introduce the standard soln. Nos. 3 and 4 are 8" condensers with ca  $\frac{3}{4}$ " inner tubes; 6 mm Pyrex tubing is used for all connections. No. 5 is a 24/40 joint; 7 and 10 are 7/25 joints; 8, 11, 17, 18, and 23 are 2 mm bore, well-ground, 2-way Pyrex stopcocks. Nos. 6, 9, 14, 16, and 21 are 2 mm bore, 3-way Pyrex stopcocks carefully lubricated with a minimum of grease. Use care to keep bores free of grease. No. 12 is a  $\text{CaCl}_2$  drying tube of  $\frac{3}{4}$ " Pyrex tubing 10" long having a glass wool plug at each end. ( $\text{P}_2\text{O}_5$  may be substituted for the  $\text{CaCl}_2$ .) No. 13, a  $\text{P}_2\text{O}_5$  tube to remove last traces of  $\text{H}_2\text{O}$ , has the same dimensions and construction as the  $\text{CaCl}_2$  tube, but is filled with a mixture of glass beads and the  $\text{P}_2\text{O}_5$ . No. 15 is a Hg trap and bubble counter. Have the center tube just dip below the surface of the Hg. Use  $\frac{3}{4}$ " tubing with a volume of ca 20 ml for each tube. No. 19 is a closed-end manometer to measure the vacuum of the system. No. 20 is an open-end manometer with ca 10 cm of Hg in each arm, and it is used only near the end of the reflux to bring the system to atmospheric pressure. No. 22 is an oil pump capable of producing a vacuum of at least 0.5 mm. No. 24 is an ionization chamber, of ca 2.8 liters' capacity. It consists of a brass cylinder, 6" in height and diameter, provided with a vacuum-tight brass inlet tube near top and brass outlet tube near bottom. Use care that no solder gets into the chamber. The wall of the chamber should be grounded. The electrode is a 1/16" brass wire, insulated from the chamber by being threaded thru a tapered amber plug and con-

ected with a short lead to both the charging device and the leaf or fibers of the electroscope or electrometer. No. 25 may be either a sensitive electroscope or an electrometer. Either the leaf- or filament-type electroscope may be used, or in the case of an electrometer the single- or double-filament type. To make the electrical system as sensitive as possible, have the electroscope or electrometer mounted as close to the electrode as possible, preferably on top of the ionization chamber. There should also be provided an electronic rectifying device capable of maintaining a potential of 200–500 volts. The negative terminal is connected by means of a suitable switch to the electrode of the chamber and to the charging terminal of the leaf or fibers of the electroscope.

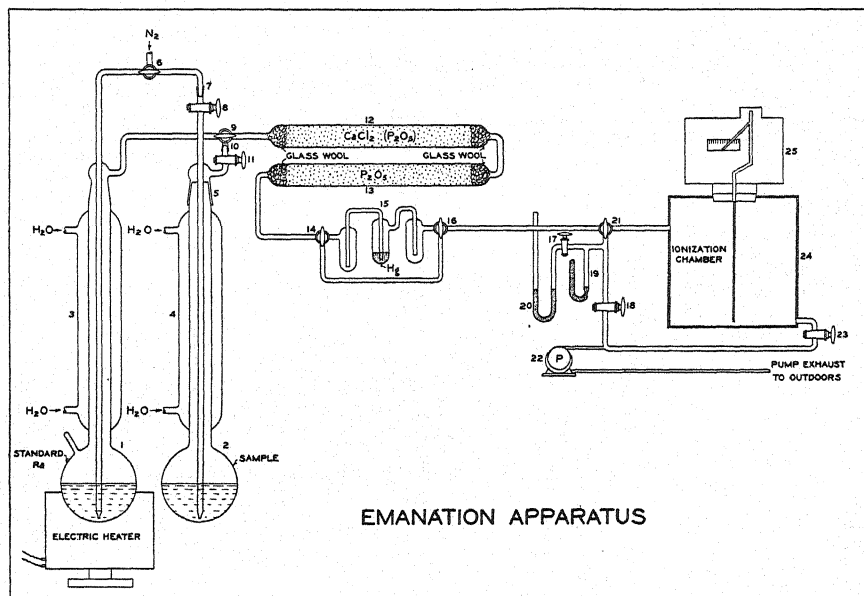


FIG. 51.—APPARATUS FOR DETERMINING RADIOACTIVITY BY EMANATION METHOD

#### 4

#### PREPARATION OF SAMPLE

Run all samples in acid soln (either HCl or HNO<sub>3</sub>) and have them free from SO<sub>4</sub> and SiO<sub>2</sub>. The soln should be clear and limpid and contain no precipitate or suspended matter.

##### A. Samples completely soluble in acids:

(1) *Solid or semi-solid form.*—Add 50 ml of HNO<sub>3</sub> (1+9) and boil for several minutes. If a residue remains, add 50 ml of HCl (1+9) and again boil. (This treatment should not be applied to samples containing grease, such as face creams, the physical appearance of which will indicate that they are insoluble in aqueous solns.)

(2) *Liquid form (clear liquid, turbid liquid, or liquid containing suspended matter).*—Add 50 ml of HNO<sub>3</sub> (1+9) to 1–10 ml of sample, boil for several minutes, and examine carefully for opalescence. If a portion of the sample remains undissolved, add 50 ml of HCl (1+9) and again boil. If clear and limpid solns are obtained, proceed as directed under (c) *Final preparation of clear solns.*

*B. Samples wholly or partly insoluble in acids:*

*(a) Preliminary treatment:*

(1) *Solids*.—If the sample is not in powder form, grind to a fine powder; ignite a weighed portion in a porcelain dish in a muffle at dull red heat, avoiding fusion; and proceed as directed under (b) *Treatment of ash*.

(2) *Semi-solids*.—Ignite quite rapidly in a muffle a weighed portion of the sample contained in a porcelain dish, avoiding fusion. (Heating too slowly or heating in the open may cause the sample to creep over edge of dish.) Proceed as directed under (b) *Treatment of ash*.

(3) *Liquids immiscible with H<sub>2</sub>O*.—Evaporate a weighed or measured portion of the sample to dryness, or as nearly so as possible, on a steam bath, and dry carefully on a hot plate. Ignite the residue in a muffle, avoiding fusion. Proceed as directed under (b) *Treatment of ash*.

(4) *Liquids containing material insoluble in HNO<sub>3</sub> (1+9)*.—Digest sample or a suitable portion of it with HNO<sub>3</sub> (1+9). Filter into a 300 ml Florence flask, and wash residue thoroly with hot H<sub>2</sub>O. Proceed as directed under (b) *Treatment of ash*, beginning "Ignite washed residue in Pt dish . . ."

*(b) Treatment of ash:*

(1) Digest the ash obtained under (a) with HNO<sub>3</sub> (1+9) on steam bath. Filter into Florence flask and wash thoroly with hot H<sub>2</sub>O. (A flask of 300 ml capacity is usually most suitable, even if it is necessary to concentrate the filtrates by boiling.) Ignite washed residue in Pt dish and cover residue with a few ml of H<sub>2</sub>O and 5–10 ml of HF. Evaporate to dryness on steam bath. Add H<sub>2</sub>O and a few ml of the HNO<sub>3</sub>, digest on steam bath, filter into Florence flask, and wash with H<sub>2</sub>O. Ash the filter paper in a Pt dish and add 5–10 ml of H<sub>2</sub>O and 1 ml of the HNO<sub>3</sub>. Examine carefully for any insoluble material; if none is found add the soln directly to the Florence flask, rinsing dish several times with H<sub>2</sub>O and adding washings to flask. Proceed as directed under (c) *Final preparation of clear solns*.

(2) If an insoluble residue that does not contain BaSO<sub>4</sub> remains, proceed as follows: Ignite insoluble residue in Pt dish and fuse with 5–10 times its weight of a fusion mixture consisting of equal weights of K<sub>2</sub>CO<sub>3</sub> and anhydrous Na<sub>2</sub>CO<sub>3</sub>. Cool, and cover with cover-glass. Neutralize the fused mass with HNO<sub>3</sub> (1+9), using a drop of phenolphthalein soln to note when the soln is acid. Heat on steam bath, add a few ml excess of the HNO<sub>3</sub>, and boil carefully. Filter soln into the Florence flask and wash thoroly. Ignite insoluble residue in Pt dish and proceed as directed under (b) *Treatment of ash*, beginning "cover residue with a few ml of H<sub>2</sub>O and 5–10 ml of HF."

(3) If insoluble residue contains appreciable quantities of BaSO<sub>4</sub>, proceed as follows: Ignite insoluble residue in Pt crucible, mix, and fuse with 5–10 times its weight of a fusion mixture consisting of equal weights of K<sub>2</sub>CO<sub>3</sub> and anhydrous Na<sub>2</sub>CO<sub>3</sub>. Cool, boil residue with a little H<sub>2</sub>O until thoroly disintegrated, and filter. Since this soln contains SO<sub>4</sub> do not mix with the acid filtrate obtained under (b) (1). Wash the residue with hot, normal Na<sub>2</sub>CO<sub>3</sub> soln until filtrate gives no test for SO<sub>4</sub>, and then with a little H<sub>2</sub>O. Dissolve washed residue (Ba-RaCO<sub>3</sub>) carefully with HNO<sub>3</sub> (1+1). If a clear solution results, combine with original acid filtrate. If an insoluble residue remains, proceed as directed under (b) (1), beginning "Ignite washed residue, etc." Combine with original acid filtrates.

*(c) Final preparation of clear solns:*

Evaporate the clear acid solns obtained under (a) or (b) to dryness in Pt dish. Add 10 ml of HCl (1+4) and again evaporate to dryness. Repeat the HCl evaporation.

Take up residue in 25 ml of the HCl, warm, and filter into a 200 ml volumetric flask, washing dish and paper well with hot H<sub>2</sub>O until volume in flask is 100–125 ml. Add 40 ml of 10% BaCl<sub>2</sub> soln and make to volume. A clear limpid soln should result.

## 5

## DETERMINATION

Keep stopcock (17) closed at all times except when system is at or near atmospheric pressure. With stopcocks 9 and 17 closed and 14, 16, and 21 open, evacuate system to a pressure of ca 1 mm as shown on manometer 19. Close stopcock 18, shut off pump, and allow system to stand 1 hour. The pressure should remain less than 5 mm.

(1) *Introducing Standard.*—Place into flask 1, thru attached tube, an accurately measured quantity of standard soln that contains 1 millimicrogram of Ra. Dilute to ca 200 ml with a boiled soln of 2% HCl containing 2% BaCl<sub>2</sub>. Seal off the tube.

(2) *Introducing Sample.*—Introduce into flask 2, a subdivision of the clear soln of sample obtained, (c), that will produce an accurately measurable increase in the rate of discharge of the electroscope, by disconnecting the flask and condenser at joint 5, and adjust to ca 200 ml with the HCl-BaCl<sub>2</sub> soln. Then lubricate the joint with P<sub>2</sub>O<sub>5</sub> at its outer periphery, leaving joints 7 and 10 dry, and assemble apparatus, taking care to have the joints tight.

(3) *De-emanation of Standard.*—Manipulate stopcocks 6, 9, 14, 16, 18, and 21 so that a gentle stream of N<sub>2</sub> (ca 3 bubbles per second as shown on the Hg trap) passing thru the soln in flask 1 will carry the radon thru the pump exhaust to outdoors without entering the ionization chamber. Heat soln to boiling with electric heater and reflux 20–25 min. in gentle stream of N<sub>2</sub>. Remove electric heater, close cock 6 first and then 9, noting exact time that stopcock 9 is closed. This seals off the standard in flask 1, and as the soln cools the slight vacuum set up in this portion of the system minimizes loss of emanation.

(4) *De-emanation of Sample.*—Treat sample in flask 2 exactly as directed for the standard, taking care that stopcocks 6 and 9 are not turned, so that flask 1 is connected to the system during the refluxing of sample in flask 2. After sample has refluxed 20 min. close stopcocks 6 and 8 and then stopcock 11, and note exact time of closing the latter. Thus the de-emanated sample is sealed in flask 2 by stopcocks 8 and 11. Then detach this portion of the apparatus at joints 7 and 10 and connect another empty sample flask and condenser unit on at joints 7 and 10.

(5) *Radon Accumulation.*—Allow both standard and sample to stand sealed 2–30 days. As may be seen in Table 25, XLIII, ca 0.3 of the equilibrium quantity of radon is formed in 2 days, 0.5 in 4 days, and practically complete equilibrium in 30 days. The sensitivity of the determination is therefore to a large degree controlled by period of storage of sample.

(6) *Background or Natural Leak.*—Connect electroscope and electrode with charging device so that a negative potential of ca 300 volts (or potential necessary to set the leaf or fibers to the maximum scale reading) is maintained on the electrode and the scope. Flush system by passing a gentle stream of N<sub>2</sub> thru empty sample flask 2, the system, and ionization chamber for 30 min. Then close stopcock 8 and evacuate system to 1 mm pressure. Close stopcocks 18 and 23, shut off pump, and fill ionization chamber with N<sub>2</sub> at atmospheric pressure. Make final adjustment of pressure with manometer 20.

Remove source of negative potential from electrode and note time that leaf or fibers pass the nearest division. Note exact time necessary for the leaf or fibers to travel across one division and then allow the system to stand until the leaf or fibers travel across the major portion of the scale, noting time necessary. To obtain the natural leak, calculate rate of discharge of instrument in "divisions/minute."

(7) *Radon in Standard.*—Immediately after making final reading for natural leak determine radon content of standard by transferring accumulated radon in standard soln from flask 1 to the ionization chamber as follows:

Evacuate the ionization chamber and apparatus to stopcock 9, taking care that manometer 20 is shut off from system. Charge electroscope and electrode negatively and during refluxing maintain electrode at maximum negative potential, as indicated on electroscope. When system is evacuated to ca 1 mm of Hg, as indicated on manometer 19, manipulate stopcocks 6 and 9 very cautiously so that the radon is carried in a slow stream of  $N_2$  thru Hg bubble trap into evacuated chamber. Bring soln to boil and so adjust the rate of  $N_2$  bubbling thru that at end of 20 min. refluxing the pressure within ionization chamber is slightly less than atmospheric pressure, as indicated on manometer 20. (The gas velocity of the radon-nitrogen gas mixture during refluxing is ca 2–4 bubbles/second as indicated in Hg bubble trap and the  $N_2$  flow is ca 1–2 bubbles/second as shown in flask.) At end of 20 min. refluxing, remove heat under flask 1 while  $N_2$  is still bubbling thru the standard soln. As soon as pressure in ionization chamber is equal to that of the atmosphere, shown on manometer 20, close stopcock 6 and then 9 to seal this part of the system off again to allow another accumulation of radon for future standardizations, and note exact time of closing stopcock 9.

Allow the mixture of radon and nitrogen in ionization chamber to stand 3 hours to allow the radon to form equilibrium quantities of radium A, B, and C before taking readings. Remove source of negative potential from electrode and electroscope exactly 3 hours after stopcock 9 is closed, and take readings of electroscope with aid of stop-watch over same range as was used for natural leak determination.

After completing the readings, pump out ionization chamber and rinse 2 or 3 times with pure  $N_2$  by evacuating and refilling ionization chamber, passing the  $N_2$  thru empty sample flask 2.

(8) *Redetermination of Natural Leak.*—Allow system to stand at least 2 hours and redetermine the background of electroscope as directed previously.

(9) *Radon in Sample.*—Disconnect empty sample flask 2 at joints 7 and 10 and replace with sealed flask 2, which contains the sample, after lubricating joint 10 with  $P_2O_5$ . For transfer of radon from sample to ionization chamber and measurement of ionization, proceed as directed under (7).

(10) *Calculations.*—Subtract natural leak of the electroscope in terms of divisions/minute from rate of fall in divisions/minute when the radon from standard soln was in the chamber. In a sample sealed for less than 30 days calculate the radon content of the standard from Table 25, XLIII. In a sample sealed for 30 days or more, the radon content is substantially equivalent to the radium content. Divide the calculated quantity of radon by the acceleration of the rate of discharge of the electroscope due to this amount of radon. The quotient will be the millimicrograms of Ra equivalent to an acceleration of one division/minute in rate of discharge of electroscope.

Subtract the natural leak of electroscope in divisions/minute from accelerated rate of fall due to the radon of the sample. Multiply this difference, which is the net effect of the radon alone in the sample, by the millimicrograms of Ra that will cause an increase of 1 division/minute as found in the standardization. If sample has been allowed to stand 30 days, the result will be the quantity of Ra in the sub-sample taken for analysis. If sample has stood less than 30 days, calculate the Ra content from Table 25, XLIII. Report result in millimicrograms of Ra per ml or per g.

*Gamma Ray Method<sup>2</sup>—Tentative.*

6

## APPARATUS

A cylindrical metal chamber (1) of ca 1000 ml capacity, which is hermetically sealed. The axis of the cylinder is vertical. On the inside is the Wulf 2-fiber system, which is fastened to an amber insulator (4), and which can be charged with the aid of an electronic rectifier charging device or a suitable charging rod. The rate of movement of the fibers is determined by means of a microscope (2). (See Fig. 52.)

7

## PREPARATION OF SAMPLE

(a) *Effective radioactivity.*—In the case of devices and preparations in which radioactive material is not ingested, but applied externally, determine the effective radioactivity directly upon sample without removing it from container in which it is to be used.

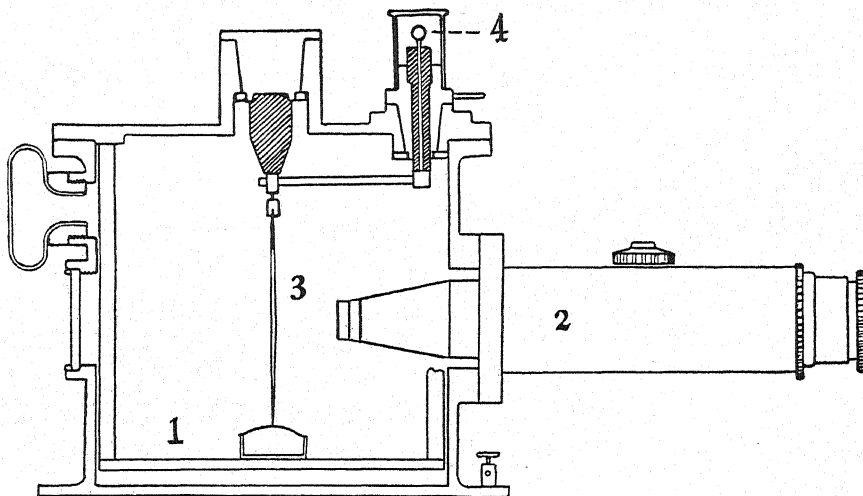


FIG. 52.—GAMMA RAY ELECTROSCOPE

(b) *Total radioactivity.*—Hermetically seal entire sample or one or more subdivisions in a suitable container, such as test tube or flask. Allow to stand at least 30 days.

8

## STANDARDS

Use known quantities of Ra measured by National Bureau of Standards.

9

## STANDARDIZATION OF ELECTROSCOPE

(a) *Natural leak.*—Charge electroscope thru charging rod by means of a charging device, to bring the fibers to a suitable point, for example at 70° division mark, after charging rod (4) is grounded. As the natural leak of the electroscope in a room free from Ra is very small, use a Ra standard to adjust the fiber approximately to desired division mark. Remove standard from room and record time when fiber crosses exact division mark. Allow electroscope to remain charged overnight. Again record time when one of the fibers crosses an exact division mark. Calculate rate of travel of fiber in seconds per division and designate the figure obtained as the natural leak (R) of the electroscope for the particular determination.



(b) *Constant*.—Place a suitable Ra standard containing 10–1000 micrograms of Ra at an exact measured distance from center of electroscope. Charge electroscope and record average time, measured by stop-watch, for at least 6 trials, of fiber to travel over that part of scale used in obtaining the natural leak. Calculate rate of travel in seconds per division and corrected time (T) due to Ra alone by following formula:

(1)  $T = PQ/Q - P$ , in which  $P$  = observed time and  $Q$  = natural leak.

Then calculate the constant (K) by following formula:

(2)  $K = ST/(R)^2$ , in which  $S$  = micrograms of Ra in the standard;  $T$  = corrected time found in (1); and  $R$  = distance between the center of electroscope and the standard.

To obtain a reliable average figure for this constant, calculate  $K$ , placing the Ra standard at different distances from the center of electroscope. Use several different standards of known Ra content.

## 10

## DETERMINATION

Place sample at suitable distance from center of electroscope. Charge electroscope as directed above, using if convenient a Ra standard to adjust fiber. Record average time taken by fiber to travel between exact division marks over that part of the scale used for the standardization. If sample contains sufficient radioactivity to permit, take average readings when it is placed at different distances from center of electroscope; if it contains only a relatively small quantity of radioactivity, fasten it with rubber bands to circumference of electroscope so as to obtain maximum ionization. Calculate micrograms of Ra ( $S'$ ) or its equivalent in terms of Ra by following formula:

(3)  $S' = K(R')^2/T'$ , in which  $K$  = constant of electroscope;  $R'$  = exact distance between center of electroscope and center of sample; and  $T'$  = corrected time in seconds per division due to radioactivity only in sample.

## SELECTED REFERENCES

<sup>1</sup> Rev. Sci. Instruments, 4, 216 (1933); 6, 99 (1935); Phy. Rev., 55, 931 (1939).

<sup>2</sup> J. Assoc. Official Agr. Chem., 19, 101 (1936).

## XXXIX. DRUGS

### SAMPLING<sup>1</sup>—TENTATIVE

1

#### *I. Tablets and Pills*

(a) *Bulk lots*.—Mix the lot as thoroly as possible without mutilating the contents. Count, weigh, and powder thoroly at least 100 units. Calculate average weight per unit.

(b) *Containers of 1000 or more units*.—Open and cautiously mix entire contents without mutilation and divide into 2 parts. Take one part (usually  $\frac{1}{2}$  or  $\frac{1}{4}$  of sample is sufficient) for analysis. Return remainder to container as a reserve sample. Count, weigh, and powder analyst's subdivision as directed under (a).

(c) *Containers of 100–500 units*.—If more than one container is available, count, weigh, and powder entire contents of one of them. If only one container is available, but there is sufficient material to warrant subdividing, proceed as directed under (b); otherwise count, weigh, and powder entire contents.

(d) *Small containers, e.g., tubes of hypodermic tablets*.—Choose a number of containers that will constitute a satisfactory sample; count, weigh, and powder contents.

(e) *Tablets or pills of small dosages, e.g., 1/100 grain of active ingredient*.—The number of units necessary may be so large as to make powdering unnecessary. A half or whole bottleful may be required. Count the units to be used but do not powder them.

#### *II. Soft Capsules*

Count and weigh a representative number of capsules and ascertain gross weight per capsule. Open capsules and transfer as much of contents as possible to weighing bottle. Clean capsules (cutting in two if necessary) and wash by agitating with alternate portions of alcohol and ether. (A few drops of glacial acetic acid mixed with the alcohol aids in the cleaning.) Finally remove the ether before a fan or air blast. Deduct weight of cleaned, empty capsules from gross weight and calculate average net contents.

#### *III. Ampuls*

Before opening ampuls dislodge any liquid adhering in neck. Mark with file or other suitable instrument the level of the liquid on the necks of requisite number of ampuls, open them near the tip, transfer bulk of contents to small flask, and mix. To determine volume of contents, wash and dry empty ampuls and fill to mark with H<sub>2</sub>O from a graduated pipet or buret.

### ACETANILID AND ACETOPHENETIDIN<sup>2</sup> (PHENACETIN)

2

#### *Qualitative Test for Acetophenetidin—Tentative*

To 0.001–0.002 g of sample in test tube add a drop of glacial acetic acid, 0.5 ml of H<sub>2</sub>O, and 1 ml of 0.1 N I soln; warm mixture to ca 40° and add a drop of HCl. If acetophenetidin alone is present, its periodide separates almost immediately in the form of reddish brown leaflets or needle-like crystals. If sample consists largely of acetanilid, separation takes place on cooling and shaking the liquid. In the presence of considerable acetanilid, the periodide first separates as minute, oily globules, which on vigorous shaking gradually become crystalline. By this test as little as 0.0005 g of acetophenetidin, if alone, may be detected in the form of its characteristic periodide.

## Quantitative Methods—Tentative

## 3

## REAGENTS

(a) *Purified iodine*.—Use reagent quality I, or dissolve 2 parts of resublimed I and 1 part of KI in 1 part of  $H_2O$ , pour the clear soln into large volume of  $H_2O$ , filter, and wash the finely precipitated I several times on perforated plate with  $H_2O$ . Dry in air and finally in desiccator containing  $H_2SO_4$ . Store in glass-stoppered weighing bottle.

(b) *Standard sodium thiosulfate soln*.—Dissolve 30 g of  $Na_2S_2O_3 \cdot 5H_2O$  in recently boiled, cooled  $H_2O$  and dilute to ca 1 liter. Standardize this soln against the purified I as follows: Weigh accurately ca 0.3 g of the purified I in small glass capsule provided with closely fitting glass cap or stopper. Place capsule in a 200 ml Erlenmeyer flask containing 0.5 g of KI dissolved in 1–2 ml of  $H_2O$ . After complete soln dilute with 10 ml of  $H_2O$  and titrate with the  $Na_2S_2O_3$  soln, using 1 or 2 drops of starch indicator VI, 3(e).

(c) *Standard iodine soln*.—Dissolve 40 g of KI in least possible quantity of  $H_2O$ , add 30 g of I, and after solution dilute to ca 1 liter. Standardize against the standard  $Na_2S_2O_3$  soln.

## 4

## DETERMINATION

(a) *Acetophenetidin* (1) Volumetric.—Place 0.2 g of the acetanilid-acetophenetidin mixture in a 50 ml lipped Erlenmeyer flask, add 2 ml of glacial acetic acid, heat gently over wire gauze to complete soln, and dilute with 40 ml of  $H_2O$  previously warmed to  $70^\circ$ . Transfer the clear liquid with two 10 ml portions of warm ( $40^\circ$ )  $H_2O$  to glass-stoppered, 100 ml volumetric flask containing 25 ml of the standard I soln warmed to  $40^\circ$ . Stopper, mix thoroly by rotating the liquid, add 3 ml of HCl, continue rotating the liquid until crystallization begins, and then set aside to cool. (If ratio of acetophenetidin to acetanilid is equal to or greater than unity, crystalline scales will form almost immediately on the addition of acid. As the proportion of acetanilid increases, however, the periodide tends to remain in the liquid state. Gentle agitation or rotation of flask in  $H_2O$ , warmed not to exceed  $40^\circ$ , hastens the formation of crystals.) When the contents are at room temp., fill flask with  $H_2O$  to within 2 or 3 ml of mark, mix thoroly by rotating mixture, and allow to stand overnight. Fill to mark with  $H_2O$ , mix thoroly, allow to stand 30 min., and filter thru a 5.5 cm dry, closely fitted filter into a 50 ml volumetric flask, rejecting ca 15 ml of first runnings but reserving it for recovery of acetanilid. Transfer the 50 ml aliquot to a 200 ml Erlenmeyer flask and titrate excess I with the standard  $Na_2S_2O_3$  soln. The formula of the precipitated periodide is  $(C_2H_5O \cdot C_6H_4NH \cdot COCH_3)_2HI \cdot I_4$ . 1 ml of 0.25 N I = 0.0224 g of  $C_{16}H_{13}O_2N$ .

(2) Gravimetric.—Filter off the periodide, preferably by suction; wash with 10–15 ml of the standard I soln; and transfer the precipitate, together with filter and any particles of precipitate remaining in volumetric flask, to a separator, using not over 50 ml of  $H_2O$ . Remove both free and added I with a few small crystals of  $Na_2SO_3$  and extract the liquid with three 50 ml portions of  $CHCl_3$ , washing each portion subsequently in a second separator with 5 ml of  $H_2O$ . After washing and clearing, filter the  $CHCl_3$  soln thru a small dry filter into a 200 ml Erlenmeyer flask, distil most of the  $CHCl_3$ , transfer residual soln (5–10 ml) by means of a little  $CHCl_3$  to a small weighed beaker, evaporate to dryness on steam bath, cool, and weigh.

(b) *Acetanilid*.—If combined weight of the acetanilid-acetophenetidin mixture is known, determine weight of acetanilid by difference; or determine it directly from a second aliquot of the filtrate from the acetophenetidin periodide (a) as follows:

Pipet 25–30 ml of the clear liquid into a separator, decolorize with solid  $Na_2SO_3$ ,

and add solid  $\text{NaHCO}_3$  in slight excess, then 1 or 2 drops of acetic anhydride. Extract with three 60 ml portions of  $\text{CHCl}_3$ , passing the  $\text{CHCl}_3$  soln thru small, dry filter into a 200 ml Erlenmeyer flask, and distil the  $\text{CHCl}_3$  by the aid of gentle heat to ca 20 ml. Add 10 ml of  $\text{H}_2\text{SO}_4$  (1+9) and digest on steam bath until residue has been reduced one-half. Add 20 ml of  $\text{H}_2\text{O}$  and continue digestion for an hour. Add a second 20 ml portion of  $\text{H}_2\text{O}$  and 10 ml of  $\text{HCl}$  and titrate very slowly, dropwise, with standard bromide-bromate soln, 5(a), until a faint yellow color remains. While adding this reagent, rotate flask sufficiently to agglomerate the precipitated tribromoaniline. 1 ml of 0.1  $N$   $\text{KBrO}_3 = 0.00225$  g of  $\text{C}_6\text{H}_5\text{ON}$ .

If the preparation contains caffeine or antipyrine, or both, in addition to acetanilid and acetophenetidin, proceed as follows: (1) Digest mixture by heating with the  $\text{H}_2\text{SO}_4$  (1+9) to convert acetophenetidin and acetanilid to phenetidin and aniline sulfates, respectively, 7(a); (2) separate caffeine and antipyrine by extraction with  $\text{CHCl}_3$ ; (3) regenerate acetophenetidin and acetanilid by treating the soln of the corresponding sulfates with solid  $\text{NaHCO}_3$  in slight excess and a few drops of acetic anhydride, and extract with  $\text{CHCl}_3$ .

#### ACETANILID AND CAFFEINE<sup>3</sup>—OFFICIAL

5

##### REAGENTS

(a) *Standard bromide-bromate soln.*—Dissolve 14 g of  $\text{KBrO}_3$  and 55 g of  $\text{KBr}$  in  $\text{H}_2\text{O}$ . Dilute to 1 liter and standardize against recrystallized and dried acetanilid, by one of following procedures: (1) Proceed as directed under 4(b), beginning "Add 10 ml of  $\text{H}_2\text{SO}_4$  (1+9)"; (2) transfer 10 ml of the soln to a glass-stoppered flask and add 25 ml of  $\text{H}_2\text{O}$ , 5 ml of 16.5%  $\text{KI}$  soln, and 5 ml of  $\text{HCl}$ . Shake thoroly and titrate the liberated  $\text{I}$  with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  soln, using starch soln as indicator, VI, 3(e).

(b) *Iodine soln.*—Dissolve 2 g of  $\text{I}$  and 6 g of  $\text{KI}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml. Treat all corks used in the distillation with  $\text{CHCl}_3$ .

6

##### PREPARATION OF SOLUTION

(a) If the sample is already in powder, rub thoroly in a mortar and keep in tightly corked tube or flask. Powders in paper, cachet, or capsule containers are frequently of such fineness as to require little further trituration except to produce a uniform product. With tablets and pills, determine their average weight and powder in a mortar. Weigh 0.3–0.5 g of sample or, if preferred, a quantity equal to, or multiple of, the average unit dose (previously ascertained by weighing collectively 20 or more such doses). Transfer to separator, add 50 ml of  $\text{CHCl}_3$  and 20 ml of  $\text{H}_2\text{O}$ , shake vigorously, and after clearing draw off lower layer thru small dry filter into a 200 ml Erlenmeyer flask. Repeat extraction twice, using 50 ml portions of  $\text{CHCl}_3$  for each extraction. Recover any caffeine-acetanilid mixture observable about apex of delivery tube of separator, edge of filter, and tip of separator by careful washing with  $\text{CHCl}_3$  and add these washings to main portion. Distil combined  $\text{CHCl}_3$  extracts to ca 10 ml.

If caffeine is present, as the free alkaloid or in other readily extractable form, the extraction may, if preferred, be made on filter paper by washing with successive 5–10 ml portions of  $\text{CHCl}_3$  (30–50 ml is usually sufficient) until extraction is complete, as indicated by absence of any residue after evaporation of a small portion of the last washing.

(b) With dilute alcoholic solns, evaporate a measured quantity on steam bath until most of alcohol has been expelled, or take an aliquot of residue from an alcohol determination and transfer to separator by pouring and rinsing with minimum quantity of  $\text{H}_2\text{O}$  so that final volume does not greatly exceed 20 ml. In order to

avoid any loss of acetanilid by hydrolysis during evaporation, add a little solid  $\text{NaHCO}_3$  and a drop of acetic anhydride. Should preparation contain other alkaloids, acidify with a few drops of  $\text{H}_2\text{SO}_4$  (1+9) immediately after acetylation to retain such basic material in the aqueous soln. Add 50 ml of  $\text{CHCl}_3$ , shake vigorously, and after clearing draw off the  $\text{CHCl}_3$  layer thru a filter into 200 ml Erlenmeyer flask. Repeat extraction twice, using 50 ml portions of  $\text{CHCl}_3$  for each extraction, and distil combined  $\text{CHCl}_3$  washings to volume of ca 10 ml.

## 7

## DETERMINATION

(a) *Caffeine*.—Treat the  $\text{CHCl}_3$  soln obtained, 6, with 10 ml of  $\text{H}_2\text{SO}_4$  (1+9) and digest on steam bath until contents of the flask are reduced to 5 ml. Add 10 ml of  $\text{H}_2\text{O}$  and continue digestion until liquid is again reduced to 5 ml. (Diluting and evaporating must be repeated until odor of acetic acid can no longer be detected in vapors.) Cool, and transfer to separator with minimum of  $\text{H}_2\text{O}$ . (Final volume should not greatly exceed 20 ml.) Add 50 ml of  $\text{CHCl}_3$ , extract in usual way, and after clearing withdraw lower layer thru a small, dry filter into a 200 ml Erlenmeyer flask. Repeat extraction with two 50 ml portions of  $\text{CHCl}_3$ . Distil combined extracts to ca 10 ml, finally transferring residual liquid, by washing with  $\text{CHCl}_3$ , to weighed beaker or crystallizing dish. Allow soln to evaporate spontaneously, or by gentle heat and an air blast, to apparent dryness. Cool, and allow to stand in open until weight becomes constant.

From preparations containing powdered cinnamon, celery seed, ginger, or other vegetable products,  $\text{CHCl}_3$  extracts, in addition to caffeine and acetanilid, certain oils, fats, waxes, resins, pigments, and other substances. After the caffeine-acetanilid mixture has been digested, these oils, etc., appear either in suspension or soln and contaminate the caffeine. Remove any suspended impurities by filtering thru small, moistened filter immediately after hydrolysis and prior to extraction with  $\text{CHCl}_3$ . Should recovered caffeine be deeply colored or contaminated with foreign matter, purify as follows: Dissolve in  $\text{H}_2\text{SO}_4$  (ca 5 ml of 0.2 *N* acid for every 100 mg of caffeine); filter, if necessary, thru moistened filter; add 1 ml of 9 *N*  $\text{H}_2\text{SO}_4$  and sufficient iodine reagent, 5(b), to color supernatant liquid a deep claret; stir, and allow to stand an hour, preferably in refrigerator. Filter and wash the periodide with a few ml of *I* soln; transfer both filter and precipitate to separator, using not more than 20 ml of  $\text{H}_2\text{O}$ ; and decolorize with a crystal of  $\text{Na}_2\text{SO}_3$ . Extract with three 50 ml portions of  $\text{CHCl}_3$  and proceed as directed above.

(b) *Acetanilid*.—(1) Transfer the soln of aniline sulfate remaining in separator to the Erlenmeyer flask used in effecting hydrolysis and heat 10 min. on steam bath to expel all traces of  $\text{CHCl}_3$ . Wash filter that was used in drying the  $\text{CHCl}_3$  soln of caffeine with 5 ml of  $\text{H}_2\text{O}$ , adding washings to main soln of aniline sulfate. Add 10 ml of  $\text{HCl}$  and titrate with the standard bromide-bromate soln, 5(a), until a faint yellow coloration remains, rotating flask sufficiently to agglomerate the precipitated tribromoaniline. 1 ml of 0.1 *N*  $\text{KBrO}_3 = 0.00225$  g of  $\text{C}_6\text{H}_5\text{ON}$ .

(2) Add an excess of the standard bromide-bromate soln to soln of aniline sulfate obtained under (b) and titrate the excess with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  after addition of 5 ml of *KI* soln and starch soln as indicator, VI, 3(e). 1 ml of 0.1 *N* bromide-bromate soln = 0.00225 g of acetanilid.

(c) *Other ingredients*.—To determine  $\text{NaHCO}_3$  also, which often appears as the  $\text{CHCl}_3$ -insoluble residue, titrate such residue with standard acid, using methyl orange indicator. The bicarbonate may also be determined by igniting the original sample (if talc is absent) or the  $\text{CHCl}_3$ -insoluble residue, with  $\text{H}_2\text{SO}_4$  and weighing resulting  $\text{Na}_2\text{SO}_4$ .

Should the "acetanilid compound" be combined with NaBr, the bromide, in the absence of other halides, may be determined volumetrically as directed under XII, 37. 1 ml of 0.1 *N* AgNO<sub>3</sub> = 0.01029 g of NaBr.

#### ACETANILID, CAFFEINE, AND CODEINE<sup>1</sup>—OFFICIAL

8

##### PREPARATION OF SOLUTION

Transfer to separator one or more average unit doses (ca 0.2 g of acetanilid) of the powdered sample; add 20 ml of H<sub>2</sub>O, 50 ml of CHCl<sub>3</sub>, and 10 drops of H<sub>2</sub>SO<sub>4</sub> (1+9); and extract in the usual way. After clearing, wash the solvent in a second separator with 5 ml of H<sub>2</sub>O and transfer to a 200 ml Erlenmeyer flask. Repeat the extraction with two 50 ml portions of CHCl<sub>3</sub>, finally distilling the combined CHCl<sub>3</sub> soln by gentle heat to ca 10 ml. Test for complete extraction.

9

##### DETERMINATION

(a) *Acetanilid and caffeine*.—Treat the CHCl<sub>3</sub> residue, 8, as directed under 7.

(b) *Codeine*.—Combine the wash H<sub>2</sub>O used in second separator under 8 with the soln of codeine sulfate. Add an excess of solid NaHCO<sub>3</sub>, extract with 5 successive portions of 30, 25, 20, 15, and 10 ml of CHCl<sub>3</sub>, wash the combined CHCl<sub>3</sub> extracts with 5 ml of H<sub>2</sub>O in second separator, and pass thru dry filter into a 200 ml Erlenmeyer flask. Test for complete extraction. Distil by gentle heat to ca 5 ml. Transfer the CHCl<sub>3</sub> soln to a small weighed beaker, evaporate to apparent dryness on steam bath, add a few drops of alcohol and a like quantity of H<sub>2</sub>O to the amorphous residue and evaporate again. Finally cool and allow the usually crystalline product to stand until the weight becomes constant. Check this result volumetrically by dissolving the residue in 3–5 ml of neutral alcohol and titrating with 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to a faint red color, using methyl red indicator, 82(b). 1 ml of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> = 0.00598 g of C<sub>18</sub>H<sub>21</sub>O<sub>3</sub>N, or 0.00634 g of C<sub>18</sub>H<sub>21</sub>O<sub>3</sub>N·H<sub>2</sub>O.

The quantity of codeine found by weight will usually be slightly greater than that determined by titration. To insure greatest possible accuracy in volumetric operations, check strength of the standard acid used by titration against pure codeine.

#### ACETANILID, CAFFEINE, AND QUININE<sup>2</sup>—OFFICIAL

10

##### PREPARATION OF SOLUTION.—See 8.

11

##### DETERMINATION

(a) *Acetanilid and caffeine*.—See 7.

(b) *Quinine*.—Combine the wash H<sub>2</sub>O used in second separator under 8 with the soln of quinine bisulfate, add a slight excess of NH<sub>4</sub>OH, and extract with three 50 ml portions of CHCl<sub>3</sub>. Wash each portion with 5 ml of H<sub>2</sub>O in a second separator and pass thru a dry filter into a 200 ml Erlenmeyer flask. Distil by gentle heat to ca 5 ml, evaporate on steam bath to apparent dryness, dissolve the amorphous alkaloid in 5 ml of neutral alcohol, and titrate with 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to a yellow color, using 2 drops of bromocresol purple indicator, 102. Heat on steam bath until most of alcohol has been expelled, adding, if necessary, sufficient 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to maintain the acid reaction. 1 ml of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> = 0.007565 g of quinine (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>N<sub>2</sub>·3H<sub>2</sub>O) or 0.007825 g of quinine sulfate, (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>N<sub>2</sub>)<sub>2</sub> H<sub>2</sub>SO<sub>4</sub>·2H<sub>2</sub>O, or 0.007934 g of quinine hydrochloride, C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>N<sub>2</sub>·HCl·2H<sub>2</sub>O, or 0.007943 g of quinine dihydrochloride, C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>N<sub>2</sub>·2HCl.

If the mixture contains acetophenetidin in place of acetanilid, proceed as outlined above, except to make the separation of caffeine and acetophenetidin as directed under 17.

ACETANILID, CAFFEINE, QUININE, AND MORPHINE<sup>3</sup>—OFFICIAL

12

## PREPARATION OF SOLUTION

Transfer to separator a quantity of the powdered sample (containing not less than 0.016 g of morphine) and add 20 ml of H<sub>2</sub>O and 10 drops of H<sub>2</sub>SO<sub>4</sub> (1+9). Extract with three 50 ml portions of alcohol-free CHCl<sub>3</sub>, wash each portion in a second separator with 5 ml of H<sub>2</sub>O, and add combined washings to the alkaloidal soln in first separator. Filter the CHCl<sub>3</sub> extracts thru small, dry filter into a 200 ml Erlenmeyer flask and distil by gentle heat to ca 10 ml.

13

## DETERMINATION

(a) *Acetanilid and caffeine*.—Proceed as directed under 7, using the CHCl<sub>3</sub> extract obtained under 12.

(b) *Quinine*.—Add to the soln of quinine and morphine sulfates obtained under 12, 4–5 ml of a 10% NaOH soln and extract with four 40 ml portions of CHCl<sub>3</sub>. Wash each portion with 5 ml of H<sub>2</sub>O and pass the clear solvent thru small, dry filter into a 200 ml Erlenmeyer flask. Remove solvent by gentle distillation and titrate residual quinine with 0.02 N H<sub>2</sub>SO<sub>4</sub> as directed under 11(b). (If the morphine salt present is contaminated with codeine, the latter will be separated and titrated with the quinine.)

(c) *Morphine*.—Wash filter used under (b) with 5 ml of H<sub>2</sub>O and add washings to the aqueous, alkaline soln of the alkaloid. Add 0.5 g of NH<sub>4</sub>Cl (or a quantity slightly in excess of that required to free the morphine as well as convert all NaOH to NaCl), 45 ml of CHCl<sub>3</sub>, and 5 ml of alcohol. Extract in usual way, washing solvent in second separator with 5 ml of H<sub>2</sub>O. Pass the CHCl<sub>3</sub> thru small, dry filter into a 200 ml Erlenmeyer flask. Repeat extraction with three 40 ml portions of CHCl<sub>3</sub>, washing and filtering as before. Collect all solvent in an Erlenmeyer flask and distil to ca 10 ml. Transfer with CHCl<sub>3</sub> to small beaker and evaporate to apparent dryness. Dissolve the residue in 1–2 ml of warm, neutral methyl alcohol; add a drop of the methyl red indicator, and titrate with 0.02 N H<sub>2</sub>SO<sub>4</sub> to a faint red color. Evaporate most of the alcohol on steam bath and, if necessary, add from a buret sufficient of the 0.02 N acid to maintain the faint red color. To insure greatest possible accuracy, check strength of standard acid used by titration against pure morphine. 1 ml of 0.02 N H<sub>2</sub>SO<sub>4</sub> = 0.0057 g of C<sub>17</sub>H<sub>19</sub>O<sub>3</sub>N.

\*NOTE: In the various operations involving fixation and subsequent liberation of morphine by means of fixed alkali and NH<sub>4</sub>Cl, the most careful attention should be paid to the manner of adding the reagents, since any undue excess of either might nullify the entire procedure. Any large excess of NaOH would naturally require for its reduction a correspondingly large quantity of NH<sub>4</sub>Cl, the latter in turn yielding its equivalent of hydroxide, relatively large quantities of which, thru interaction with NaCl, tend to inhibit any permanent liberation of alkaloid and thus prevent complete extraction. Furthermore, NH<sub>4</sub>Cl in large quantity operates retentively on the morphine in soln, due in part possibly to the formation of an alkaloidal hydrochloride.

ACETANILID AND SODIUM SALICYLATE<sup>4</sup>—OFFICIAL

14

## PREPARATION OF SOLUTION

For tablets and pills, ascertain their average weight and powder in mortar. Weigh quantity of powdered sample equal to, or multiple of, an average unit dose (ca 0.2 g of acetanilid); transfer to separator containing 10 ml of H<sub>2</sub>O; and for every unit dose add 0.1 g of solid NaHCO<sub>3</sub>. In the examination of alcoholic preparations, distil the alcohol from a measured volume on steam bath, transfer to separator with minimum quantity of H<sub>2</sub>O, and add 0.5–1.0 g of solid NaHCO<sub>3</sub>.

15

## DETERMINATION

(a) *Acetanilid*.—Extract the alkaline soln, 14, with three 50 ml portions of  $\text{CHCl}_3$ ; wash each portion with 5 ml of  $\text{H}_2\text{O}$  in second separator and collect the solvent, without previous drying, in a 200 ml Erlenmeyer flask. Reserve aqueous soln for determination of Na salicylate, (b). Distil the  $\text{CHCl}_3$  very gently to ca 5 ml, add 10 ml of  $\text{H}_2\text{SO}_4$  (1+9), and completely hydrolyze on steam bath. Proceed as directed under 7(b), beginning "Add 10 ml of  $\text{HCl}$ ."

(b) *Sodium salicylate*.—Acidify the aqueous soln of Na salicylate, (a), with a few drops of  $\text{HCl}$  and extract 3 to 5 times with 25 ml portions of  $\text{CHCl}_3$  to remove the salicylic acid. Treat each portion in second separator with 20 ml of  $\text{H}_2\text{O}$  containing 1 g of anhydrous  $\text{Na}_2\text{CO}_3$  for every 0.1 g of salicylic acid. Shake vigorously, and after clearing wash each portion again in third separator with 5 ml of  $\text{H}_2\text{O}$ . Add washings to main aqueous alkaline soln of Na salicylate. Dilute to known volume; transfer an aliquot, representing ca 0.1 g of salicylic acid, to a 200 ml Erlenmeyer flask; dilute to 60–75 ml; heat nearly to boiling; add slowly 50–80 ml of approximately 0.1 N I soln, sufficient to insure an excess during digestion; and digest for an hour on steam bath. Remove the free I with a few drops of  $\text{Na}_2\text{S}_2\text{O}_3$  soln and decant the clear liquid thru a weighed Gooch crucible, retaining most of the precipitate, tetraiodophenylenequinone  $(\text{C}_6\text{H}_2\text{I}_2\text{O})_2$ , in the flask. To the latter add 50 ml of boiling  $\text{H}_2\text{O}$ , digest 10 min. on steam bath, filter, and gradually wash all precipitate into the Gooch crucible, using for this purpose and final washing ca 200 ml of hot  $\text{H}_2\text{O}$ . Dry the precipitate to constant weight in air bath at  $100^\circ$ .  $(\text{C}_6\text{H}_2\text{I}_2\text{O})_2 \times 0.4654 = \text{NaC}_7\text{H}_5\text{O}_3$ .

Should mixture contain caffeine or antipyrine, or both, these substances will appear with the acetanilid in the first  $\text{CHCl}_3$  extract and may be determined as directed in the remarks following 43(b). Should the acetanilid be replaced by acetophenetidin in the mixture, the general procedure would not be materially altered, the acetophenetidin being weighed directly after recovery from its washed  $\text{CHCl}_3$  soln as separated from the Na salicylate. If, instead of Na salicylate, the mixture contains the free acid or its  $\text{NH}_4$  salt, add a larger quantity of  $\text{NaHCO}_3$  prior to extraction with  $\text{CHCl}_3$  to insure the fixation of salicylic acid.

In the analysis of a mixture of caffeine, acetanilid, Na salicylate, and codeine, the following procedure is recommended: (1) Extraction of caffeine, acetanilid, and salicylic acid from the acidified soln; (2) washing the  $\text{CHCl}_3$  soln with aqueous  $\text{Na}_2\text{CO}_3$  soln for recovery of the salicylic acid, preliminary to its treatment with I soln; (3) separation of caffeine and acetanilid as directed under 7(a) and 7(b); and (4) recovery of codeine from the soln of its sulfate after treatment with  $\text{NaHCO}_3$  and  $\text{CHCl}_3$ .

ACETOPHENETIDIN (PHENACETIN) AND CAFFEINE<sup>7</sup>—TENTATIVE

16

## PREPARATION OF SOLUTION

In preparations containing acetophenetidin instead of acetanilid, but otherwise identical, make the gross separation of the caffeine-acetophenetidin mixture as directed under 6.

17

## DETERMINATION

(a) *Caffeine*.—Treat the  $\text{CHCl}_3$  extract, 16, with 10 ml of  $\text{H}_2\text{SO}_4$  (1+9) and digest on steam bath until the liquid is reduced to ca 5 ml. Dilute with 10 ml of  $\text{H}_2\text{O}$  and continue digestion until volume is again reduced to 5 ml; again add 10 ml of  $\text{H}_2\text{O}$  and continue heating until residual liquid amounts to 8–10 ml. Repeat the diluting and evaporating until odor of acetic acid can no longer be detected in the



vapors. If, during digestion, particles of acetophenetidin remain on sides of flask, rinse them into the soln with a few drops of  $\text{CHCl}_3$ . (Great care must also be given to the degree of evaporation. Should the aqueous-acid soln and suspension of caffeine-acetophenetidin be concentrated much beyond the limits indicated, more or less phenetidin sulfonate is likely to be formed, which later resists acetylation and conversion to acetophenetidin.) Cool, transfer with  $\text{H}_2\text{O}$  to separator so that the final volume does not greatly exceed 20 ml, and proceed as directed under 7(a).

(b) *Acetophenetidin*.—Wash the filter used to dry the  $\text{CHCl}_3$  with 5 ml of  $\text{H}_2\text{O}$ , receiving the washings in the separator containing the soln of phenetidin sulfate. Treat with successive small portions of solid  $\text{NaHCO}_3$  until, after complete neutralization of free acid, an excess of  $\text{NaHCO}_3$  remains. Add 50 ml of  $\text{CHCl}_3$ , and for every 0.1 g of acetophenetidin known or believed to have been present, 5 drops of acetic anhydride. Shake vigorously, allow to clear, and withdraw the  $\text{CHCl}_3$  into second separator containing 5 ml of  $\text{H}_2\text{O}$ . Shake this mixture, and after clearing pass the solvent thru small, dry filter into 250 ml Erlenmeyer flask. Repeat extraction twice with 50 ml portions of  $\text{CHCl}_3$ , washing each portion with the 5 ml of  $\text{H}_2\text{O}$  in the second separator. Distil the combined  $\text{CHCl}_3$  extractions to ca 10 ml, transfer residual soln with sufficient fresh solvent to a weighed 50 ml beaker or crystallizing dish, evaporate on steam bath to apparent dryness, and finally remove any considerable excess of acetic anhydride by repeated additions and evaporations of 1 ml of  $\text{CHCl}_3$  and a drop of alcohol. (The reformed acetophenetidin should finally appear as a whitish, crystalline mass with a faint, acetous odor that disappears completely on standing some hours in the open or over lime in a vacuum desiccator.) Weigh at intervals until final weight differs from preceding by not more than 0.0005 g.

#### ACETOPHENETIDIN (PHENACETIN) AND SALOL<sup>3</sup>

18

##### *Acid Hydrolysis Method—Tentative*

(a) *Acetophenetidin*.—Weigh on a tared 5.5 cm filter a quantity of sample equal to, or multiple of, the average weight of a unit dose and wash with sufficient successive small portions of  $\text{CHCl}_3$  to extract completely all acetophenetidin and salol present in the mixture (ca 40 ml). Collect soln in weighed 100 ml beaker and evaporate on warm plate (50–60°) to apparent dryness, using air blast. Let stand 24 hours at room temp. to practically constant weight and weigh. By means of  $\text{CHCl}_3$  transfer the crystalline residue to 50 ml lipped Erlenmeyer flask, evaporate solvent by means of air blast and gentle heat, add 10 ml of  $\text{H}_2\text{SO}_4$  (1+9), and evaporate on steam bath until volume is reduced one-half. Add 10 ml of  $\text{H}_2\text{O}$  and continue digestion as before. Add a second 10 ml of  $\text{H}_2\text{O}$  and evaporate to 5 ml. Transfer residue with ca 20 ml of  $\text{H}_2\text{O}$  to a small separator and extract the salol with 15, 10, and 5 ml of  $\text{CHCl}_3$ , washing each extract with 5 ml of  $\text{H}_2\text{O}$  in a second separator. Add the wash water in second separator to solution of phenetidin sulfate in first separator and proceed as directed under 17(b), beginning, "Treat with successive small portions of solid  $\text{NaHCO}_3$ ."

(b) *Salol*.—Subtract weight of acetophenetidin from combined weight of the two ingredients to obtain weight of salol.

19

##### *Alkaline Hydrolysis Method—Tentative*

(a) *Acetophenetidin*.—On a small, tared filter or in a small beaker weigh a quantity of the sample containing not more than 0.08 g of salol; exhaust with  $\text{CHCl}_3$  as directed under 18(a); collect solvent in small lipped Erlenmeyer flask; and evaporate the  $\text{CHCl}_3$  by means of air blast without heat. Add 10 ml of 2.5%  $\text{NaOH}$

soln and heat 5 min. on steam bath. Cool quickly to room temp. in running  $\text{H}_2\text{O}$ . Transfer the liquid to a separator with minimum quantity of  $\text{H}_2\text{O}$  and rinse flask with first 20 ml portion of  $\text{CHCl}_3$  to be used in the following extraction. Extract the alkaline soln with three 20 ml portions of  $\text{CHCl}_3$ ; wash each portion in a second separator with 5 ml of  $\text{H}_2\text{O}$ , and pass the  $\text{CHCl}_3$  soln thru a small, dry filter into a 200 ml Erlenmeyer flask. Reserve combined alkaline soln and washings for determination of salol (b). Distil combined  $\text{CHCl}_3$  extracts to ca 5 ml. Transfer by means of a little  $\text{CHCl}_3$  to small, weighed beaker or crystallizing dish, evaporate on steam bath with the aid of air blast, cool, and weigh residual acetophenetidin at intervals until weight becomes constant.

(b) *Salol*.—Place the reserved combined alkaline soln and washings (a) in a 500 ml iodine flask, dilute with  $\text{H}_2\text{O}$  to ca 200 ml, run in from a buret an excess (ca 50 ml) of 0.1 *N* bromide-bromate soln, 26(c), add 10 ml of  $\text{HCl}$ , and shake 1 min., then at intervals for 30 min. Add 10 ml of 15%  $\text{KI}$  soln and shake at intervals for 15 min. Titrate the free  $\text{I}$  with standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln previously standardized against the 0.1 *N* bromide-bromate soln. 1 ml of 0.1 *N* bromide-bromate soln = 0.001784 g of salol.

#### ACETYLSALICYLIC ACID<sup>a</sup>

20

##### *Melting Point—Official*

If excipients are present, treat 0.2–0.3 g with small portions of  $\text{CHCl}_3$  and filter into a beaker or evaporating dish. Evaporate the bulk of the  $\text{CHCl}_3$  on steam bath and complete by spontaneous evaporation until thoroly dry. Determine melting point of crystalline residue by U.S.P. method.

#### FREE SALICYLIC ACID

21

##### *Qualitative Test—Official*

Shake 0.5 g sample in small Erlenmeyer flask with ca 10 ml of  $\text{CHCl}_3$  and filter. Evaporate, treat residue with 10 ml of cold  $\text{H}_2\text{O}$ , and filter. Add 1 drop of a 10%  $\text{FeCl}_3$  soln. Only a very faint violet color should result.

##### *Quantitative Method—Official*

22

#### REAGENTS

(a) *Standard salicylic acid soln*.—Dissolve 0.01 g of salicylic acid in 100 ml of alcohol. Use only a freshly prepared soln.

(b) *Ferric ammonium sulfate soln*.—Add 1 ml of normal  $\text{HCl}$  to 2 ml of 8%  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  soln and dilute with  $\text{H}_2\text{O}$  to 100 ml.

23

##### PREPARATION OF SAMPLE.—See 1.

24

#### DETERMINATION

In each of two colorimeter tubes mix 48 ml of  $\text{H}_2\text{O}$  and 1 ml of the freshly prepared  $\text{FeNH}_4(\text{SO}_4)_2$  soln. Shake 2.5 g of the powdered sample with exactly 25 ml of alcohol and filter if necessary. Immediately add 1 ml of the filtrate to one of the colorimeter tubes and 1 ml of the standard salicylic acid soln to the other, and mix. Immediately and rapidly make color comparisons and calculate the free salicylic acid on the basis of the acetylsalicylic acid present. If color is too intense for satisfactory comparison, repeat entire determination, using smaller weight of the powdered sample.

## TOTAL SALICYLATES

25

*Iodine Method<sup>9</sup>—Official*

Weigh 0.1 g sample into 200 ml Erlenmeyer flask, add 20 ml of  $H_2O$  and 1 g of  $Na_2CO_3$ , and heat on steam bath 15 min. Filter, if necessary, to remove talc. Dilute to 60–75 ml, heat nearly to boiling, add slowly an excess (50–80 ml) of approximately 0.1 N I soln, and proceed as directed under 15(b) (sodium salicylate). Multiply weight of precipitate by 0.4015 to obtain total salicylic acid and deduct the free salicylic acid, 24. Multiply remainder by 1.304 to obtain weight of acetylsalicylic acid.

*Bromine Method—Official*

26

## REAGENTS

- (a) *Sodium hydroxide soln.*—Dissolve 2 g of NaOH in  $H_2O$  and dilute to 100 ml.
- (b) *Potassium iodide soln.*—Dissolve 20 g of KI in  $H_2O$  and dilute to 100 ml.
- (c) *Standard bromide-bromate soln (0.1 N bromine soln).*—Dissolve 3 g of  $KBrO_3$  and 12 g of KBr in  $H_2O$  and dilute to 1 liter. Standardize 30 ml of the Br soln by transferring to iodine flask and adding 25 ml of  $H_2O$ , 5 ml of the KI soln, and 5 ml of HCl. Shake thoroly. Titrate with 0.1 N  $Na_2S_2O_3$  soln, using starch indicator, VI, 3(e).

27

## DETERMINATION

Saponify 0.5 g sample with 10 ml of the NaOH soln by heating 15 min. on steam bath. Dilute with  $H_2O$  in volumetric flask to 500 ml. Transfer aliquot of this soln, representing not less than 0.04 g nor more than 0.05 g of acetylsalicylic acid, to a 500 ml iodine flask; add 30 ml of the standard bromide-bromate soln and 5 ml of HCl and immediately insert stopper. Shake repeatedly 30 min. and allow to stand 15 min. Remove stopper just sufficiently to introduce quickly 5 ml of the KI soln, taking care that no Br vapors escape, and immediately stopper flask. Shake thoroly, remove stopper, and rinse it and neck of flask with a little  $H_2O$  so that washings flow into flask. Titrate with 0.1 N  $Na_2S_2O_3$  soln, using starch indicator, VI, 3(e). 1 ml of 0.1 N bromide-bromate soln = 0.0023 g of salicylic acid, or 0.003 g of acetylsalicylic acid.

*Double Titration Method for Acetylsalicylic Acid<sup>10</sup>—Official*

28

## PREPARATION OF SOLUTION

(a) *Dry extraction method (applicable in all cases).*—Treat a weighed quantity of sample containing not less than 0.3 g of acetylsalicylic acid with small portions of  $CHCl_3$ , filter into a beaker, and wash residue with  $CHCl_3$  until completely extracted. Evaporate bulk of the  $CHCl_3$  on steam bath, finishing with the aid of electric fan without heat.

(b) *Wet extraction method (applicable in absence of acids and alkalies, or alkaline earth carbonates).*—Transfer the accurately weighed sample to a small separator containing ca 20 ml of  $H_2O$ . Shake out repeatedly with  $CHCl_3$ , using successively 30, 25, 20, 15, 10, and 5 ml portions, and test for completeness of extraction by evaporating a portion of final extraction on watch-glass. Filter combined  $CHCl_3$  portions thru cotton and wash funnel and cotton with  $CHCl_3$ . Evaporate bulk of the  $CHCl_3$  on steam bath, finishing with aid of electric fan without heat.

(c) *Acetylsalicylic acid and uncoated tablets containing no excipient.*—Dissolve the sample directly in 10 ml of neutral alcohol.

29

## DETERMINATION

Dissolve the dry  $\text{CHCl}_3$  extract in 10 ml of neutral alcohol and titrate immediately and rapidly with 0.1 *N* alkali soln, using phenolphthalein indicator. Use the first persistent pink color as the end point, since any slight excess of alkali has a tendency to hydrolyze the ester quickly. Add a volume of the 0.1 *N* alkali equal to that used in the first titration and then add 5 ml more. Heat on steam bath 15 min. Titrate back with 0.1 *N* acid. If product is pure, the total quantity of alkali consumed will be twice that of first titration. Each ml of 0.1 *N* alkali consumed in the 2 titrations = 0.009 g of acetylsalicylic acid.

30

COMBINED ACETIC ACID IN ACETYLSALICYLIC ACID<sup>11</sup>—OFFICIAL

If excipients are present weigh accurately 2 g of the powdered material and transfer to a separator, using ca 25 ml of  $\text{H}_2\text{O}$ . Extract completely with  $\text{CHCl}_3$ , testing last extraction by evaporating small quantity of the  $\text{CHCl}_3$  to dryness. (Usually 6 extractions with 30, 25, 20, 10, 10, and 5 ml portions of  $\text{CHCl}_3$  are sufficient.) Filter the  $\text{CHCl}_3$  fractions thru pledget of cotton into a beaker. Wash original beaker, funnel, and cotton with  $\text{CHCl}_3$  and add these washings to the  $\text{CHCl}_3$  soln in beaker. Evaporate the  $\text{CHCl}_3$  on steam bath; dry residue at  $80^\circ$  for 15 min.

Treat the  $\text{CHCl}_3$  extract, or if no excipients are present, 2 g of the powdered material, in a 150 ml beaker with 30 ml of *N* NaOH and evaporate on steam bath nearly to dryness. Transfer to a separator, using 10 ml of  $\text{H}_2\text{O}$ , 20 ml of 10%  $\text{H}_2\text{SO}_4$ , and finally two 5 ml portions of  $\text{H}_2\text{O}$ . Extract with successive portions of  $\text{CHCl}_3$ , using first fraction of 50 ml to rinse the beaker in which the saponification was carried on. Continue the extractions with  $\text{CHCl}_3$  until all salicylic acid is removed (ca 6 extractions). During these extractions keep stopper in separator to guard against loss of acetic acid by evaporation. Collect the  $\text{CHCl}_3$  fractions in second separator, wash with 25 ml of  $\text{H}_2\text{O}$ , and wash this  $\text{H}_2\text{O}$  once with 5 ml of  $\text{CHCl}_3$ . Discard the  $\text{CHCl}_3$  extractions and return the wash  $\text{H}_2\text{O}$  to the acid  $\text{H}_2\text{O}$  in first separator. Transfer the acid  $\text{H}_2\text{O}$  containing acetic acid and  $\text{H}_2\text{SO}_4$  to 200 ml volumetric flask, wash separators thoroly with  $\text{H}_2\text{O}$ , add to flask, dilute to volume, and mix thoroly. Pipet two 50 ml portions, using same pipet and draining it same length of time. Place one portion in a receptacle suitable for titration and the other in a large Pt dish. Titrate the first portion at once with 0.5 *N* alkali, using phenolphthalein indicator. Evaporate the portion in the Pt dish on steam bath to dryness, take up in 10 ml of  $\text{H}_2\text{O}$ , and again evaporate, repeating this process twice more. (During evaporation guard against contact with  $\text{NH}_3$  vapors.) Take up residue in  $\text{H}_2\text{O}$  and titrate with 0.5 *N* alkali, using phenolphthalein indicator. Subtract the second titration reading from the first and calculate percentage of acetic acid on a 0.5 g sample. 1 ml of 0.5 *N* alkali = 0.03002 g of acetic acid.

31 ACETYLSALICYLIC ACID IN MIXTURES CONTAINING ACETOPHENETIDIN AND CAFFEINE<sup>12</sup>—OFFICIAL

Ascertain average weight of a number of tablets and reduce to fine powder.

Weigh ca 0.2 g of the powder, transfer to separator with ca 25 ml of  $\text{H}_2\text{O}$ , and extract carefully with repeated portions of  $\text{CHCl}_3$ . Test final extraction by evaporating a small portion on steam bath to dryness; ca 6 extractions are generally required, and these can be made with 30, 25, 20, 10, 10, and 5 ml portions of  $\text{CHCl}_3$ . Collect the  $\text{CHCl}_3$  fractions in a separator and draw off into a 200 ml Erlenmeyer flask, placing pledget of cotton in stem of separator to filter the  $\text{CHCl}_3$ . Wash separator twice with 5 ml portions of  $\text{CHCl}_3$ , passing this thru the cotton and leaving any  $\text{H}_2\text{O}$  that may have separated in the separator. Add the  $\text{CHCl}_3$  washings to flask and evaporate the  $\text{CHCl}_3$  on steam bath to volume of ca 2 ml. Add 10 ml

of  $\text{H}_2\text{SO}_4$  (1+9), connect with reflux condenser, and digest 30 min., partially immersing flask in boiling water bath. Cool, and transfer to separator, rinsing condenser with  $\text{CHCl}_3$  and using minimum quantity of  $\text{H}_2\text{O}$  to effect transfer, so that final volume does not greatly exceed 20 ml. Extract the caffeine and salicylic acid with 6 portions of  $\text{CHCl}_3$ , using 30, 25, 20, 15, 10, and 10 ml. Collect these fractions in a separator, add 20 ml of  $\text{H}_2\text{O}$  and 1 g of  $\text{Na}_2\text{CO}_3$ , and shake thoroly. Drain off the  $\text{CHCl}_3$  into another separator and wash twice more with 15 and 10 ml of  $\text{H}_2\text{O}$ . Reject the  $\text{CHCl}_3$  and combine the  $\text{Na}_2\text{CO}_3$  soln and wash waters in a 200 ml Erlenmeyer flask. Heat on steam bath to expel traces of  $\text{CHCl}_3$ , dilute to 100 ml with  $\text{H}_2\text{O}$ , then add slowly 25–40 ml of strong I soln (about 0.2 N), sufficient to insure excess during digestion, and digest 1 hour on steam bath. Remove free I with a few drops of  $\text{Na}_2\text{S}_2\text{O}_3$  soln. Decant clear soln thru a weighed Gooch, retaining most of precipitate in flask. To latter add 50 ml of boiling  $\text{H}_2\text{O}$ , digest 10 min. on steam bath, filter, and wash gradually all the precipitate into the Gooch, using altogether ca 200 ml of hot  $\text{H}_2\text{O}$  to complete the operation. Dry to constant weight in air bath at  $100^\circ$  and weigh the precipitate of tetraiodophenylenequinone  $(\text{C}_6\text{H}_2\text{I}_2\text{O})_2$ . Weight of precipitate  $\times 0.4016$  = total salicylic acid present. If free salicylic acid is present, deduct from the total; the difference  $\times 1.304$  = weight of acetylsalicylic acid.

#### ACETYSALICYLIC ACID, ACETOPHENETIDIN, AND CAFFEINE<sup>18</sup>—TENTATIVE

##### 32

##### REAGENTS

(a) *Sulfuric acid*.—2%. Pour ca 6.0 ml of  $\text{H}_2\text{SO}_4$  into 500 ml of  $\text{H}_2\text{O}$ .

(b) *Sodium bicarbonate soln*.—Use freshly prepared. Add 3 g of  $\text{NaHCO}_3$  to 45 ml of  $\text{H}_2\text{O}$  previously cooled to  $15^\circ$  or lower. Stir until dissolved and add 2–3 drops of 10%  $\text{HCl}$ .

##### 33

##### DETERMINATION

(a) *Acetylsalicylic acid*.—Make determination as soon as possible to prevent any hydrolysis in the  $\text{NaHCO}_3$  soln.

Weigh sufficient powdered sample to represent at least 0.04 g of caffeine, transfer to a separator containing ca 10 ml of  $\text{H}_2\text{O}$  cooled to  $15^\circ$  or lower, and shake thoroly. Add 15 ml of the cooled  $\text{NaHCO}_3$  soln slowly to prevent mechanical loss due to effervescence and immediately extract with successive portions of  $\text{CHCl}_3$ . Wash each portion of  $\text{CHCl}_3$  thru a second separator containing 2 ml of the cold  $\text{NaHCO}_3$  soln and filter thru cotton moistened with  $\text{CHCl}_3$ . (Extraction is complete when a final shakeout evaporated to dryness leaves a negligible residue. Usually 5 extractions with ca 30 ml portions of  $\text{CHCl}_3$  are sufficient.) Set aside combined  $\text{CHCl}_3$  extracts containing caffeine and acetophenetidin for later treatment. Transfer wash  $\text{H}_2\text{O}$  in second separator to soln in first separator, rinsing several times with small portions of  $\text{H}_2\text{O}$ . Acidify combined  $\text{NaHCO}_3$  solns with  $\text{HCl}$  (1+1) and extract the acetylsalicylic acid by shaking with successive portions of  $\text{CHCl}_3$ , filtering each portion thru a funnel containing pledget of cotton moistened with  $\text{CHCl}_3$  (usually 5 extractions are sufficient). Evaporate combined  $\text{CHCl}_3$  extracts on steam bath with aid of a fan or gentle air blast until volume is ca 10 ml. Transfer to suitable small tared container with the aid of  $\text{CHCl}_3$  and evaporate to dryness by means of fan or gentle air blast without heat. Dry in desiccator overnight and weigh as acetylsalicylic acid. The extracted acetylsalicylic acid may be checked by the bromine method or by the double titration method (26 or 28).

(b) *Acetophenetidin and caffeine*.—Evaporate the  $\text{CHCl}_3$  soln containing the acetophenetidin and caffeine on steam bath and transfer, when volume reaches 5–10 ml, to a 100 ml beaker by means of small portions of  $\text{CHCl}_3$ . Evaporate again to volume of ca 5 ml and add 10 ml of 2%  $\text{H}_2\text{SO}_4$ . Introduce a stirring rod and heat

mixture on bath until all the  $\text{CHCl}_3$  has evaporated, stirring occasionally. Cool to room temp. and decant thru a tared Gooch crucible previously dried to constant weight at  $100^\circ$  (no suction required). Collect filtrate in 150 ml beaker, retaining as much of the acetophenetidin as possible in beaker. Rinse sides of beaker containing the acetophenetidin with 5–10 ml of  $\text{CHCl}_3$ , add 10 ml of 2%  $\text{H}_2\text{SO}_4$ , and heat on bath as before until all  $\text{CHCl}_3$  has evaporated. Cool, and decant thru same crucible as before. Repeat process with another 10 ml portion of the  $\text{H}_2\text{SO}_4$ , and finally wash the acetophenetidin quantitatively into the crucible with  $\text{H}_2\text{O}$ . Wash beaker and crucible with  $\text{H}_2\text{O}$  until filtrate measures ca 75 ml. Dry crucible at  $100^\circ$  and weigh the acetophenetidin.

To the filtrate containing the caffeine and the small amount of acetophenetidin that went into soln (ca 0.075 g), add 8 ml of  $\text{H}_2\text{SO}_4$  (1+10) and evaporate on steam bath to volume of ca 10 ml. Transfer by means of small portions of  $\text{H}_2\text{O}$  to a 50 ml Erlenmeyer flask previously marked for volumes of 5 and 10 ml. Proceed as directed in 16, 17, bearing in mind that the hydrolysis must be continued until no odor of acetic acid is present. The hydrolysis is hastened somewhat if the flask is allowed to hang in the steam from a wire wrapped around its neck so that the mouth of the flask is about level with the surface of the bath (ca 3 evaporations are usually sufficient). Add the weight of acetophenetidin obtained to the weight of acetophenetidin collected in the Gooch crucible to obtain the total acetophenetidin content of the sample.

#### ACETYSALICYLIC ACID, ACETOPHENETIDIN, AND SALOL<sup>14</sup>—TENTATIVE

34

##### REAGENTS

- (a) *Sodium bicarbonate soln.*—Prepare fresh as directed in 32(b).
- (b) *Standard bromide-bromate soln (0.1 N bromine soln).*—See 26(c).
- (c) *Standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln.*—0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ .
- (d) *Sodium hydroxide soln.*—2.5% w/v NaOH.

35

##### DETERMINATIONS

(a) *Acetylsalicylic acid.*—Make the determination as soon as possible to prevent any hydrolysis in the  $\text{NaHCO}_3$  soln.

Weigh 0.5–1.0 g of powdered sample and proceed as directed in 33(a).

(b) *Acetophenetidin and salol.*—Evaporate combined  $\text{CHCl}_3$  solns containing the acetophenetidin and salol [corresponding to acetophenetidin and caffeine, 33(a)] to dryness by means of gentle air blast or fan without heat. Dissolve residue in a few ml of ether and again evaporate to dryness. Treat residue as directed in 19(a), beginning “Add 10 ml of 2.5% NaOH soln.” For salol, change the procedure as follows: Transfer the alkaline salol soln, freed from the acetophenetidin, to a volumetric flask and make to volume with  $\text{H}_2\text{O}$ . Take an aliquot of this soln, containing a quantity of salol not exceeding 0.08 g, for bromination.

#### ACETYSALICYLIC ACID AND PHENOLPHTHALEIN IN TABLETS<sup>15</sup>—TENTATIVE

36

##### PREPARATION OF SAMPLE.—See 1.

37

##### DETERMINATIONS

Weigh sufficient powdered material to contain 0.05–0.1 g of phenolphthalein. Extract the dry powder repeatedly with 20 ml portions of ether and filter into a separator. Test for complete extraction (5–8 extractions required).

(a) *Acetylsalicylic acid.*—Shake the ethereal soln for at least 1 min. each time with two 20 ml portions of 4%  $\text{NaHCO}_3$  soln (temp.  $20^\circ$  or less). Transfer soln to second separator. Wash the ether with two 10 ml portions of  $\text{H}_2\text{O}$  and add to the

bicarbonate soln. Extract the bicarbonate soln with 20 ml of ether. Draw off lower aqueous layer into 100 ml volumetric flask. Wash ether with small portions of  $H_2O$ , rinse into flask, and dilute to mark. Add wash ether to bulk of solvent in original separator. Reserve ethereal soln for determination of the phenolphthalein.

Transfer an aliquot of the bicarbonate soln containing not less than 0.3 g of acetylsalicylic acid to a separator. (The acid must be isolated from the bicarbonate soln as rapidly as possible to prevent hydrolysis.) Acidify with 10%  $HCl$  and extract the liberated acetylsalicylic acid with 30, 20, 20, 10, and 10 ml portions of  $CHCl_3$ -ether solvent (3+2). Wash each extract with 2 ml of  $H_2O$  in a second separator and filter thru pledget of cotton moistened with the solvent into a counterpoised tared beaker. Test for complete extraction. Evaporate the solvent to 10–15 ml on water bath and complete evaporation without aid of heat. Dry residue to constant weight at room temp. The weight may be checked by the double titration method (28).

(b) *Phenolphthalein*.—Extract the original ethereal soln with 20 ml portions of 3%  $NaOH$  soln until all the phenolphthalein has been removed as indicated by the color. Transfer these alkaline extracts to a second separator, acidify with 10%  $HCl$ , and extract with  $CHCl_3$ -ether solvent (3+2). Wash each portion of extract in a third separator with 2 ml of  $H_2O$  to which has been added 1 or 2 drops of 10%  $HCl$ . Filter the extracts into counterpoised tared beaker, using in stem of funnel pledget of cotton moistened with the  $CHCl_3$ -ether mixture. Evaporate on  $H_2O$  bath and dry residue to constant weight at  $120^\circ$ . The weight may be checked by the tetraiodo method, 164.

#### SALICYLIC ACID IN PRESENCE OF OTHER PHENOLS<sup>16</sup>—TENTATIVE

38

##### PREPARATION OF SAMPLE

(a) *Powders*.—Weigh into a volumetric flask such a quantity of material that an aliquot of 25–50 ml will contain ca 0.13 g of phenol. If acid, make alkaline with 4%  $NaOH$ , adding 25 ml in excess, fill to mark with  $H_2O$ , and shake well.

(b) *Liquids*.—Proceed as directed under 39.

39

##### DETERMINATION

Transfer to a separator sufficient quantity of soln to represent ca 0.13 g of phenol. Acidify with 10%  $H_2SO_4$  and extract with ether, using 20, 15, 15, and 10 ml portions, until extraction is completed. Combine ether extracts in second separator. Shake with saturated  $NaHCO_3$  soln, using 15, 15, and 10 ml portions, and finally shake with 15 ml of  $H_2O$ . Combine the  $NaHCO_3$  soln and washings and extract the combined  $NaHCO_3$  extracts with 15 ml of ether. Add latter to main bulk of ether and reserve for phenol determination. Acidify the  $NaHCO_3$  soln with  $HCl$ . Extract with  $CHCl_3$ -ether (2+1), using 30, 25, 20, and 10 ml until salicylic acid is completely removed. Filter extracts into beaker thru cotton previously saturated with  $CHCl_3$ . Evaporate to 5 ml on covered steam bath with aid of electric fan, allowing last 5 ml to evaporate spontaneously. Dissolve residue in 10 ml of neutral alcohol and titrate with 0.1  $N$   $NaOH$ , using phenolphthalein as indicator. 1 ml of 0.1  $N$   $NaOH$  = 0.01381 g of salicylic acid,  $C_6H_4OHCOOH$ .

#### AMINOPYRINE (PYRAMIDON)

40

##### Qualitative Tests<sup>17</sup>—Official

(a) Dissolve 0.01 g of sample in 2 ml of  $H_2O$  and add a few drops of yellow  $HNO_3$  (containing nitrous acid). A purplish blue colored soln is produced.

(b) Dissolve 0.01 g of sample in 2 ml of  $H_2O$  and add 1 ml of 10%  $FeCl_3$  soln. A purple to violet color develops, but it becomes red on addition of  $H_2SO_4$  (1+9).

(c) Dissolve 0.1 g of sample in 2 ml of  $H_2O$  and add a few drops of 5%  $AgNO_3$  soln. After few seconds a purple to violet color is produced and on standing a deposit of metallic Ag results (useful for detecting aminopyrine in antipyrine).

(d) Dissolve 0.1–0.2 g of sample in 2 ml of  $H_2O$ , add 1 or 2 drops of 0.2% soln of  $NaNO_2$  and a few drops of  $H_2SO_4$  (1+9), and shake a few seconds. A purplish blue color develops, then gradually disappears, leaving a colorless soln. Avoid excess of  $NaNO_2$  as it destroys the color (useful for detecting antipyrine in presence of aminopyrine). On addition of a few more drops of the  $NaNO_2$  soln and the dilute  $H_2SO_4$  a yellowish green colored soln remains after the disappearance of the purple coloration if antipyrine is present.

41

*Quantitative Method*<sup>18</sup>—Official

Pulverize material in mortar and mix powder thoroly. Place 1 g of sample in a 100 ml volumetric flask, add 60 ml of normal  $H_2SO_4$ , and shake several minutes to insure complete soln of the aminopyrine.<sup>18</sup> Make up to mark with normal  $H_2SO_4$ . Filter, if not clear, thru dry filter, rejecting first part of filtrate. Pipet a 20 ml aliquot of the soln, or filtrate, into separator; make distinctly alkaline with either  $NH_4OH$  or with 5%  $NaOH$ ; and shake out with 20, 15, 10, 10, and 5 ml portions of  $CHCl_3$ . Combine the  $CHCl_3$  extracts in second separator and wash with 2 ml of  $H_2O$ . Filter the  $CHCl_3$  soln into weighed beaker thru pledget of cotton saturated with  $CHCl_3$ . Extract the wash  $H_2O$  with 5 ml of  $CHCl_3$  and add this to combined  $CHCl_3$  extracts. Evaporate combined  $CHCl_3$  extracts just to dryness on water bath with aid of electric fan and dry residue in oven at temp. of boiling  $H_2O$  for 10 min. Cool in desiccator, and weigh as aminopyrine. Identify aminopyrine by means of its melting point and qualitative tests.

ANTIPYRINE AND CAFFEINE<sup>18</sup>—OFFICIAL

42

PREPARATION OF SAMPLE

(a) Weigh a quantity of finely powdered sample equal to, or multiple of, an average unit dose; transfer to filter and extract with  $CHCl_3$  to separate caffeine and antipyrine from usual excipients of tablet and pill combinations. Distil greater part of the  $CHCl_3$  and evaporate remainder on steam bath.

(b) With alcoholic preparations, remove alcohol from a measured quantity of sample by heating on steam bath. Extract residue with three 50 ml portions of  $CHCl_3$  in a separator. Distil greater portion of the  $CHCl_3$  and evaporate remainder on steam bath.

43

DETERMINATION

(a) *Antipyrine*.—Transfer residue obtained, 42, which should weigh ca 0.25 g, to 125 ml separator by means of two 5 ml portions of alcohol-free  $CHCl_3$ , followed by 10 ml of  $H_2O$ . Add 1 g of  $NaHCO_3$  and 10–15 ml of 0.2 N I (or double quantity of 0.1 N I), adding latter in small portions and shaking mixture vigorously after each addition. (The I should then be in excess of that required to convert all the antipyrine into the mono-iodo derivative. If it is not, add a little more I and shake mixture again.) Remove free I with a small crystal of  $Na_2S_2O_3$  and add 15 ml of washed  $CHCl_3$ , shaking vigorously 1 min. After clearing, draw off the  $CHCl_3$  into second separator; wash with 5 ml of  $H_2O$ , filter thru small, dry filter into weighed 50 ml beaker, and evaporate to apparent dryness on steam bath, using air blast. Repeat extraction with 2 (3, if 0.1 N I has been used) 25 ml portions of washed  $CHCl_3$ , washing, filtering, and evaporating each portion as directed previously. Recover any crystalline product separating about tip of delivery tube, funnel, and



edge of filter by judicious washing with  $\text{CHCl}_3$ . Dry the nearly colorless, crystalline residue of caffeine and iodoantipyrine 30 min. at  $100^\circ$ , cool, and weigh. Designate this weight as "A."

The use of alcohol-free  $\text{CHCl}_3$  in connection with the iodination of antipyrine is necessary in order to preclude the formation of  $\text{CHI}_3$ , the presence of which in the composite residue A would vitiate the result.

Dissolve the composite residue in 5 ml of glacial acetic acid, add 10 ml of saturated  $\text{SO}_2$  soln, and wash with hot  $\text{H}_2\text{O}$  into 400–500 ml beaker until final volume is ca 200 ml. Add sufficient  $\text{AgNO}_3$  soln to precipitate all the I (ca 0.3 g of  $\text{AgNO}_3$ ) and a few drops of  $\text{HNO}_3$ , heat nearly to boiling, and stir to agglomerate the  $\text{AgI}$ . Add 15 ml of  $\text{HNO}_3$ , cover beaker with watch-glass, and boil gently 5 min. Decant thru weighed Gooch crucible; wash precipitate once with a little alcohol, then with two 100 ml portions of boiling  $\text{H}_2\text{O}$ ; and finally transfer  $\text{AgI}$  to the crucible. Wash several times with hot  $\text{H}_2\text{O}$  and again with alcohol to remove traces of organic matter, dry 30 min. at  $110^\circ$ , cool, and weigh. Weight of  $\text{AgI} \times 0.8014 = \text{weight of antipyrine}$ .

(b) *Caffeine*.—Multiply weight of  $\text{AgI}$  by 1.3374 and subtract product from weight "A," under (a).

In the analysis of a mixture containing caffeine, antipyrine, acetanilid, and Na salicylate, the following steps are essential in effecting a separation: (1) Extraction of caffeine, acetanilid, and antipyrine from the aqueous, alkaline soln with  $\text{CHCl}_3$ ; (2) hydrolytic treatment with  $\text{H}_2\text{SO}_4$  of the three substances thus separated preliminary to determination of caffeine and antipyrine as directed under (a).

#### BARBITAL AND PHENOBARBITAL<sup>20</sup>—OFFICIAL

(Applicable in absence of stearic acid.)

44

#### REAGENTS

(a) *Alkaline salt soln*.—Dissolve 20 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , dilute to 1 liter, add  $\text{NaCl}$  to saturation, and filter.

(b) *Solvent*.—Mix 20 ml of ether and 80 ml of  $\text{CHCl}_3$ .

45

#### DETERMINATION

Transfer 0.3 g of powdered sample to a separator and dissolve in 10 ml of the alkaline salt soln. If tablet lubricants (other than stearic acid) are present, wash with 15 ml of ether and decant from top of separator. Repeat extraction with ether twice. Add 2 ml of  $\text{HCl}$  to the alkaline soln, then 5 ml of  $\text{H}_2\text{O}$  to prevent precipitation of salt. Extract with  $\text{CHCl}_3$ -ether 5 times, using 30, 20, 20, 10, and 10 ml portions of the solvent. Test for complete extraction by extracting with an additional 10 ml of solvent and evaporating in separate beaker. Combine solvent in second separator and wash with 2 ml of  $\text{H}_2\text{O}$  acidified with a drop of  $\text{HCl}$ . Filter solvent thru pledget of cotton into a small weighed beaker. Evaporate on steam bath with aid of electric fan, heat 10 min. at  $90$ – $100^\circ$ , cool in desiccator, and weigh. Determine melting point to check purity of residue.

46

#### Alternative Method<sup>21</sup>—Official

(Applicable in presence of stearic acid.)

Dissolve residue obtained in 45, in 10 ml of alcohol, add 20 ml of saturated, aqueous soln of  $\text{Ba}(\text{OH})_2$ , and stir well. Filter into a separator and wash residue and filter with two or three 10 ml portions of the  $\text{Ba}(\text{OH})_2$  soln. Acidify the soln with 10%  $\text{HCl}$  and proceed as directed under 45, beginning "Extract with  $\text{CHCl}_3$ -ether 5 times."

47

CAMPHOR<sup>22</sup>—OFFICIAL

(Not applicable to synthetic camphor)

Weigh accurately into 400 ml round-bottomed Pyrex flask sufficient quantity of powdered material to contain ca 2 g of camphor. Add 10 ml of benzene and 10 ml of H<sub>2</sub>O and connect flask with apparatus for steam distillation. Use 8-12" bulb condenser, well cooled, the outlet of which reaches to bottom of a 200 ml flask. Distil with steam, collecting the benzene and ca 100 ml of aqueous distillate. Disconnect condenser and wash it slowly with 5 ml of alcohol from pipet in such a manner as entirely to wet inside of condenser. Wash condenser in same manner with 10 ml of benzene. Add both washings to contents of receiver. Saturate distillate with NaCl, add sufficient H<sub>2</sub>SO<sub>4</sub> (1+9) to insure acidity, transfer to separator, shake, and separate the two layers. Rinse original receiver with 10 ml of benzene and use rinsings to re-extract the aqueous soln. Separate aqueous layer and extract it once more with 10 ml of benzene. Wash combined benzene extracts with 10 ml of saturated salt soln rendered distinctly alkaline with Na<sub>2</sub>CO<sub>3</sub>. Separate layers and extract aqueous layer with 10 ml of benzene. Discard aqueous solns, transfer benzene to 50 ml volumetric flask, and make up to mark with benzene. Shake soln and filter into a 200 mm polariscope tube, using water-jacketed tube, if necessary, to maintain constant temp. of 20°. Make 10 readings, using a bichromate filter, and take average reading for calculating the camphor. Calculate quantity of camphor (*Q*) contained in the 50 ml of benzene and, therefore, in the sample taken, from average reading in circular degrees (*a*) by following formula:  $Q = 0.6171a - 0.0022a^2$ .

The value of *Q* does not vary directly with length of tube. If a longer or shorter tube than directed is used, correct value of *a* to a 200 mm tube, and then make calculation by above formula.

## MONOBROMATED CAMPHOR IN TABLETS

*Method I.<sup>23</sup>—Official*

48

## REAGENT

*Sodium amalgam.*—Cut ca 1 g of bright metallic Na into small pieces and dissolve in 100 g of warm Hg contained in small porcelain mortar by impaling the pieces successively on point of a file and holding them submerged in the Hg until the rather violent action is complete. Keep resulting amalgam in a tightly corked bottle.

49

## PREPARATION OF SAMPLE.—See 1.

50

## DETERMINATION

Weigh a portion of the powdered sample corresponding to 0.1-0.2 g of monobromated camphor, and transfer with 20 ml of alcohol and 10 ml of H<sub>2</sub>O to a small (100 ml) round-bottomed flask containing 15 g of the Na amalgam. Connect flask by means of rubber stopper with a vertical condenser. Boil mixture gently over wire gauze at least 30 min. Cool slightly and wash out condenser tube with 5 ml of alcohol and 5 ml of H<sub>2</sub>O, receiving washings in flask. Place flask on steam bath and heat for another hour, or until the evolution of H has nearly or quite ceased. Toward latter part of this operation, to facilitate reduction, render liquid about neutral with a few drops of acetic acid. Transfer contents of flask to a separator, withdrawing the Hg into second separator and washing it with at least two 50 ml portions of H<sub>2</sub>O. Pass the several aqueous solns thru small filter, collecting clear filtrate in suitable beaker. Precipitate with 10% AgNO<sub>3</sub> soln, add ca 5 ml of HNO<sub>3</sub>, and filter, collecting the AgBr in weighed Gooch crucible. Wash with H<sub>2</sub>O and alcohol, dry

at 100°, and weigh. Weight of AgBr  $\times 1.23$  = weight of monobromated camphor. Run a control on the amalgam to determine whether any correction is necessary.

*Method II.*<sup>24</sup>—Official, first action

51

PREPARATION OF SAMPLE.—See 1.

52

DETERMINATION

Weigh in small beaker a quantity of the powdered sample equivalent to ca 0.2 g of monobromated camphor, add 25 ml of alcohol, warm on steam bath, and filter into flask (preferably ca 250 ml capacity and provided with a ground-in condenser), washing both beaker and filter with warm alcohol. Add 50 ml of alcoholic KOH soln, XXXI, 24, and 25 ml of alcoholic AgNO<sub>3</sub> soln (0.2 g in 50 ml of alcohol), and connect with reflux condenser. Boil gently 1.5 hours, adding at intervals thru condenser 25 ml more of the alcoholic AgNO<sub>3</sub> soln. Cool, and transfer contents of flask to large evaporating dish. Dilute to 200 ml and decant into a beaker, washing the sediment of Ag<sub>2</sub>O with H<sub>2</sub>O by decantation. Boil the soln 5 min. with 1 g of Zn dust to clarify; filter into another beaker, washing thoroly with H<sub>2</sub>O, and add HNO<sub>3</sub> to decided acidity and 0.1 *N* aqueous AgNO<sub>3</sub> soln to complete precipitation. When the AgBr has agglutinated filter on a weighed Gooch crucible, wash with H<sub>2</sub>O and alcohol, dry at 100°, and weigh. Run a control on the reagents used. Correct for the presence of halogens if necessary. Weight of AgBr  $\times 1.23$  = weight of monobromated camphor.

METHENAMINE (HEXAMETHYLENETETRAMINE) IN TABLETS<sup>25</sup>—TENTATIVE

53

REAGENT

*Modified Nessler's reagent.*—(1) Dissolve 10 g of HgCl<sub>2</sub>, 30 g of KI, and 5 g of acacia in 200 ml of H<sub>2</sub>O, and filter thru cotton; (2) dissolve 15 g of NaOH in 100 ml of H<sub>2</sub>O; (3) mix 20 ml of soln (1) with 10 ml of soln (2).

54

PREPARATION OF SAMPLE.—See 1.

55

DETERMINATION

Weigh 0.5 g of powder into a round-bottomed flask, and add 100 ml of H<sub>2</sub>O and 25 ml of HCl (1+2.5). Connect with reflux condenser (preferably of worm type) and boil gently 15 min. Cool, wash condenser tube with a little H<sub>2</sub>O, and transfer contents of flask to 250 ml volumetric flask, finally diluting to mark. Chill 30 ml of the Nessler reagent and add a 10 ml aliquot of the hydrolyzed soln of sample. Wash neck of container with jet of H<sub>2</sub>O and allow to stand at least 1 min. Add 10 ml of acetic acid (1+1.5) in such manner that inside of neck is completely washed by the reagent, mix quickly and thoroly by rotating and tilting flask, and immediately add from a buret 20 ml of 0.1 *N* I soln. Titrate excess I with 0.1 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, adding 5–10 drops of starch indicator, VI, 3(e), toward end of operation, to disappearance of blue color. The final color of the soln is a pale straw-green. If preferred, the end point may be determined by the reappearance of a faint blue color by addition of a drop of the I soln. 1 ml of 0.1 *N* I = 0.00117 g of methenamine.

METHYLENE BLUE (METHYLTHIONINE CHLORIDE)<sup>26</sup>—OFFICIAL

56

PREPARATION OF SAMPLE

(a) *Tablets.*—Weigh separately at least 20 tablets to ascertain variation in weight. Weigh collectively all unbroken tablets and calculate average weight per tablet. Powder finely in a mortar at least 10 tablets or 5 g of methylene blue and protect from moisture in weighing bottle.

(b) *Capsules*.—Count and weigh representative number of capsules and ascertain gross weight per capsule. Open capsules and transfer as much as possible of contents to weighing bottle. To clean the gelatine capsules cut in two if necessary and wash by agitating with alternate portions of alcohol and ether. Repeat until thoroly clean, finally removing the ether before fan or air blast. A few drops of glacial acetic acid mixed with the alcohol aids in the cleaning. Deduct weight of cleaned empty capsules from gross weight and calculate average net contents.

57

## PREPARATION OF SOLUTION

(a) *Foreign material absent*.—Weigh into 50 ml beaker 0.1–0.14 g of the prepared sample, 56, and transfer to 200 ml volumetric flask with 100–140 ml of  $H_2O$ . Dissolve completely by heating on steam bath, with frequent shaking, for 30 min.

(b) *Oils or water-insoluble material present*.—Transfer to 150 ml beaker weighed quantity of prepared sample, 56, corresponding to 0.1–0.14 g of methylene blue. Add 15 ml of  $CCl_4$ , warm on steam bath a few minutes, and stir with glass rod to dissolve oils. Transfer to 100 ml separator, using ca 50 ml of hot  $H_2O$  and a little  $CCl_4$  if necessary. Cool, shake, and allow to separate. Transfer the  $CCl_4$  with the undissolved material into second separator for further treatment. (A clear aqueous soln of the dye should now remain in the first separator. If not clear, extract with another 15 ml portion of  $CCl_4$ , transferring in similar manner any remaining insoluble material to second separator.) Add ca 10 ml of  $CCl_4$  to second separator and remove the methylene blue by shaking vigorously with 20–40 ml portions of  $H_2O$  until practically no more dye is extracted. (A few drops of glacial acetic acid hastens this extraction.) To the aqueous extracts in a 400 ml beaker add main soln from first separator, cover with inverted watch-glass on glass rods, and evaporate to volume of ca 50 ml. Proceed as directed under (c). The  $CCl_4$  soln may be reserved for qualitative tests for oils.

(c) *Water-soluble material present*.—Use either the aqueous soln from (b), or a weighed portion of the sample corresponding to 0.1–0.14 g of methylene blue dissolved by heating on steam bath in 150 ml beaker with ca 50 ml of  $H_2O$  for 30 min., shaking occasionally. Transfer to 100 ml separator, keeping volume as small as possible. Extract with dichlorhydrin, using 10, 5, 3, and 2 ml portions. Combine the dichlorhydrin extracts in 200–300 ml separator, add 3 or 4 times their volume of  $CCl_4$ , and extract dye with  $H_2O$  by repeated vigorous shaking with 30–50 ml portions. A few drops of glacial acetic acid hastens the removal. From the combined aqueous extracts remove any traces of dichlorhydrin by shaking once with ca 15 ml of  $CCl_4$ , which is drawn off after settling 5–10 min. Evaporate aqueous extracts to ca 50 ml over a flame, covering beaker as in (b) with inverted watch-glass. Transfer to 200 ml volumetric flask. Dissolve completely by heating on steam bath with frequent shaking for 30 min.

58

## DETERMINATION

Conduct a blank in same manner as the determination, including filtration. Cool the soln from 57(a) or (c), add 50 ml of glacial acetic acid, shake thoroly, and allow to stand at least 25 min. Add from buret a total of 30 ml of 0.2 N I soln, adding first 10 ml by fast drops with constant rotating of the flask and the remaining 20 ml at full speed, and continue the shaking. Stopper flask and allow to stand 50 min., shaking thoroly 5 or 6 times during interval. Dilute to mark with  $H_2O$ , shake, and let stand 10 min. longer. Filter rapidly thru dry, folded, 12 cm filter paper. Titrate 100 ml aliquot with 0.1 N  $Na_2S_2O_3$  soln, with or without starch indicator as desired. Correct for number of ml required to titrate blank run in same way. 1 ml of 0.2 N I soln = 0.01495 g of methylene blue ( $C_{16}H_{18}N_3ClS \cdot 3H_2O$ ).

NITROGLYCERIN<sup>27</sup>—OFFICIAL, FIRST ACTION

59

## REAGENT

*Alcoholic potassium hydroxide.*—Dissolve 15 g of KOH in ethyl alcohol and dilute with alcohol to 100 ml.

60

## APPARATUS

(a) *Connecting bulb.*—Hopkins' style, ca 7.6 cm (3") in diameter. This style has long inlet tube with opening on side of tube.

(b) *Condenser.*—Water-cooled, length 56 cm (22"), and preferably of Pyrex glass.

(c) *Adapter tube.*—Approximately 2.25 cm ( $\frac{1}{8}$ ") in diameter at top and with narrow outlet.

(d) *Scrubber-trap.*—Any efficient trap in which all vapor is washed thoroly with H<sub>2</sub>O before it leaves distilling flask (see Fig. 53).

61

## DETERMINATION

## Method I

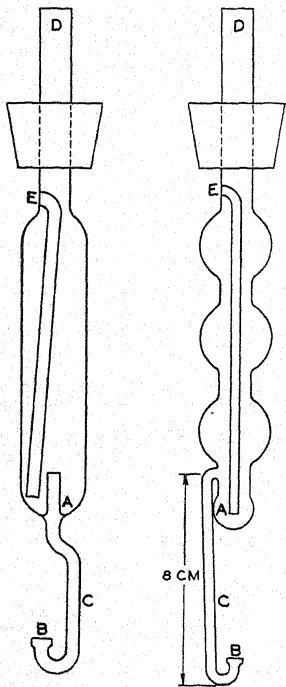


FIG. 53.—SCRUBBER TRAP FOR AMMONIA DISTILLATION

(a) Place in 50 ml beaker sufficient quantity of weighed sample to yield ca 0.0324 g of nitroglycerin. If sample consists of tablets, count those taken; if of powdered material, mix thoroly before weighing. Add 10 ml of ether, and to facilitate extraction reduce tablets to fine powder by means of glass stirring rod having flattened end. Stir thoroly. Decant the ether thru dry 7 cm quantitative filter paper into 250 ml beaker containing 10 ml of alcohol. Hold filter paper in place in funnel with the stirring rod and pour ether down rod. Make four additional extractions in same way. Dissolve the ether-insoluble residue in small quantity of H<sub>2</sub>O, transfer soln to separator, and extract it twice with 10 ml portions of ether. Filter these extracts, add them to first extracts, and evaporate combined solns to volume of ca 10 ml by means of fan.

(b) Transfer the alcoholic soln containing the nitroglycerin to 800 ml Kjeldahl flask, rinsing beaker first with 10 ml of alcohol and then with a little H<sub>2</sub>O. Dilute to ca 300 ml with recently boiled and cooled NH<sub>3</sub>-free H<sub>2</sub>O and place flask on wire gauze with asbestos center. Add 2 g of Devarda alloy (by means of funnel), ca 4 cm of heavy (ca 16 gage) Al wire, and 10–15 ml of the alcoholic KOH soln. Immediately after adding the alkali, place a little H<sub>2</sub>O in the scrubber trap (A and B), and insert into neck of flask the rubber stopper carrying scrubber trap and connecting bulb. Connect outlet tube of connecting bulb with the water-cooled condenser, which has been fixed in an upright position and fitted with an adapter tube dipping to bottom of 500 ml Erlenmeyer flask containing a measured volume (ca 25 ml) of 0.02 N acid (HCl or H<sub>2</sub>SO<sub>4</sub>) and 10–15 ml of H<sub>2</sub>O, and so inclined that tip of adapter is submerged as far as practicable under surface of liquid in flask. Heat distillation flask ca 1 hour, using small flame and regulating heat so that rapid evolution of H— but no appreciable distillation—takes place. Gradually increase heat until dis-

tillation begins; when active foaming ceases, continue distillation with large flame until all but ca 40 ml of the liquid in the distilling flask has distilled over. Lower flame toward end of distillation to avoid cracking flask. Remove receiver containing distillate, add sufficient methyl red indicator, 82(b), to make soln red, and titrate excess acid with 0.02 *N* NaOH soln. From difference between this excess and quantity added, after making such correction as may be shown to be necessary by a blank test with same quantity of reagents and distilled in same manner, calculate percentage of nitroglycerin in sample. 1 ml of 0.02 *N* acid = 0.001514 g of nitroglycerin.

62

*Method II*

Place in glass-stoppered Erlenmeyer flask sufficient sample, accurately weighed, to yield ca 0.0648 g (1 grain) of nitroglycerin. If sample consists of tablets, count those taken; if of powdered material, mix thoroly before weighing portion taken for analysis. Add 50 ml of alcohol by means of pipet. To facilitate extraction reduce tablets to fine powder with glass stirring rod flattened at one end. Stopper flask and shake. Allow mixture to settle, transfer 25 ml aliquot of clear soln to 800 ml Kjeldahl distilling flask, dilute to ca 300 ml with the  $\text{NH}_3$ -free  $\text{H}_2\text{O}$ , and proceed as directed in 61(b).

TERPIN HYDRATE IN ELIXIRS<sup>28</sup>—TENTATIVE

63

## REAGENTS

(a) *Salt soln.*—Dissolve 20 g of common salt in  $\text{H}_2\text{O}$  and make to 100 ml, or dilute 3 volumes of saturated salt soln with 1 volume of  $\text{H}_2\text{O}$ .

(b) *Alcohol-chloroform soln.*— $\text{CHCl}_3$  containing 5–7% by volume of alcohol.

64

## DETERMINATION

Measure 10 ml of sample into separator and dilute with 25 ml of the salt soln. (Quantity to be weighed should be ca 0.2 g, and the dilution with salt soln should be such as to reduce the alcoholic content to ca 10–15% by volume before extraction with alcohol- $\text{CHCl}_3$ .) Extract successively with six 15 ml portions of alcohol- $\text{CHCl}_3$ , separating the  $\text{CHCl}_3$  layer carefully each time so that none of watery layer will be carried thru with the  $\text{CHCl}_3$ . Collect all  $\text{CHCl}_3$  fractions and wash twice with the salt soln, using 15 ml for first washing and 5 ml for second. Wash each salt soln with 5 ml portion of alcohol- $\text{CHCl}_3$ , adding this portion to original  $\text{CHCl}_3$  extracts. Filter combined  $\text{CHCl}_3$  extracts containing the terpin hydrate in soln thru plug of cotton into 100 ml low-form, tared beaker, being sure that separation from salt wash  $\text{H}_2\text{O}$  is perfect. Evaporate off the  $\text{CHCl}_3$  at room temp. with aid of air blast. Use no heat in the evaporation. Wipe off beaker when  $\text{CHCl}_3$  is entirely gone, allow to stand in balance 10 min., and weigh. Do not dry in desiccator because terpin hydrate loses  $\text{H}_2\text{O}$  under these conditions. Report as g per 100 ml.

65

TERPIN HYDRATE AND CODEINE IN ELIXIRS<sup>29</sup>—TENTATIVE

(a) *Terpin hydrate.*—Measure 10 ml sample from a small buret (allow buret to drain 5 min.) into a separator. Add 10 ml of  $\text{H}_2\text{O}$  and 5 ml of 10%  $\text{H}_2\text{SO}_4$ , and immediately extract with 25 ml of petroleum benzin. Drain aqueous layer into a second separator. Wash the petroleum benzin twice with 2 ml portions of  $\text{H}_2\text{O}$  and add washings to aqueous layer. Discard the petroleum benzin. Completely extract the aqueous soln with  $\text{CHCl}_3$ -alcohol solvent (95+5), (7 extractions of 20, 10, 10, 10, 10, 10, 10 ml should be sufficient). Make an additional extraction and evaporate to dryness to test for complete extraction. Combine  $\text{CHCl}_3$ -alcohol extracts and wash with 5 ml of  $\text{H}_2\text{O}$ . Filter extract thru pledget of cotton, previously wet

with the solvent, into a tared dish. Place dish in desiccator (or similar apparatus) prepared according to Fig. 54. Adjust vacuum so incoming air just ripples surface of liquid, and evaporate to apparent dryness. Weigh residue and report as g/100 ml terpin hydrate.

(b) *Codeine*.—Measure 25 ml sample into a separator, add 25 ml of  $H_2O$  and 1 ml of 10%  $NH_4OH$  and completely extract the alkaloid with  $CHCl_3$  (6 extractions of 25, 20, 15, 10, 10, and 10 ml should be sufficient). Combine extracts in a second separator and completely extract the codeine with 10%  $H_2SO_4$  (4 extractions of 15, 10, 10, and 10 ml should be sufficient). Wash combined acid extracts with 10 ml of  $CHCl_3$  and discard solvent. Make the acid soln ammoniacal and extract 5 times with  $CHCl_3$ , using 30, 20, 20, 10, and 5 ml. Test for complete extraction of the alkaloid. (Make an additional extraction with 10 ml of  $CHCl_3$ ; evaporate the solvent in separate beaker and dissolve residue in a few drops of methyl alcohol; add a drop

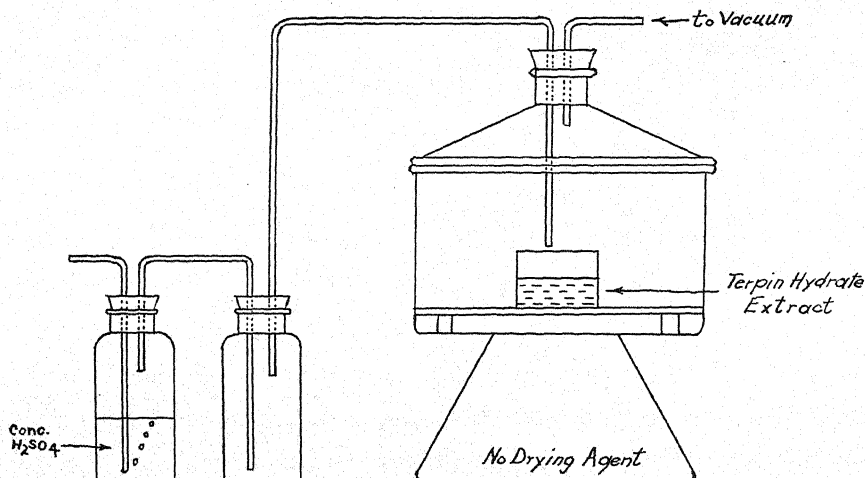


FIG. 54.—APPARATUS USED IN DETERMINATION OF TERPIN HYDRATE AND CODEINE IN ELIXIR

of methyl red indicator, 82(b), and dilute with 20 ml of  $H_2O$ , carbonate free. A yellow color indicates incomplete extraction. Titrate, and add quantity thus obtained to the total.) Combine the  $CHCl_3$  extractions in a second separator, into stem of which is inserted pledget of cotton wet with  $CHCl_3$ . Wash combined extractions with 1 ml of  $H_2O$  containing 1 drop of  $NH_4OH$  and evaporate on water bath, using electric fan to prevent decrepitation of residue. When dry, remove immediately and complete determination by one of following procedures:

(1) To the alkaloidal residue add 2–3 ml of methyl alcohol, cover beaker with watch-glass, and heat on steam bath until the residue, including any portions thereof that may adhere to the upper part of the beaker, is completely dissolved. Add 2 drops of the methyl red indicator and, without dilution with  $H_2O$ , titrate carefully with 0.02 N  $H_2SO_4$ , to a faint pink, avoiding an excess. Cover beaker and digest on steam bath until all particles are completely dissolved. If more than 2 ml of alcohol is added, evaporate excess. Cool, and dilute with 50 ml of boiled  $H_2O$  (soln should now be yellow). Finish titration with the standard acid to a faint red.

(2) Dissolve residue in 2–3 ml of methyl alcohol on steam bath. Add 2 drops of

the methyl red indicator and then add from buret 5–10 ml excess of 0.02  $N$   $H_2SO_4$ , noting total quantity used. Cover beaker with watch-glass and heat on steam bath until the residue, including any portions thereof that may adhere to the upper part of the beaker, is completely dissolved. Dilute with 50 ml of cold, previously boiled  $H_2O$ . Titrate back with the 0.02  $N$   $NaOH$  soln. The  $H_2O$  and alkali should be sufficiently free from carbonates to insure a sharp end point with methyl red. Report as g/100 ml codeine hydrate. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00634 g of  $C_{18}H_{21}O_3N \cdot H_2O$ .

66

SULFONAL AND TRIONAL<sup>20</sup>—TENTATIVE

Mix ca 0.5 g of sample with pure, clean sea sand and place mixture in a Knorr tube containing half-inch layer of asbestos. Using bell jar and vacuum, extract

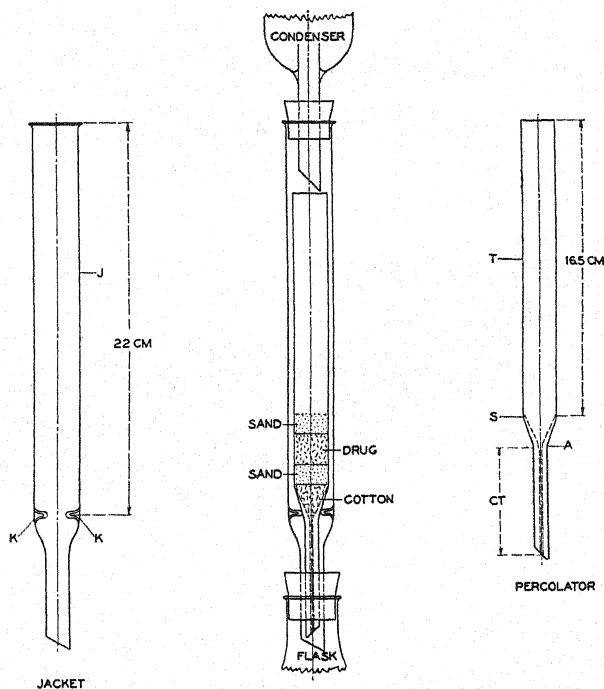


FIG. 55.—AUTOMATIC PERCOLATOR

mixture with 10 portions of 10 ml each of ether, mixing sample with sand by means of glass rod before each addition of ether. Collect ether extractions in tared flask, distil off bulk of ether, and allow remaining solvent to evaporate spontaneously, rotating flask to aid evaporation. Dry residue in desiccator over  $H_2SO_4$  18 hours and weigh. Identify residue by means of its melting point.

If desired, the extraction may be made in a suitable automatic apparatus (Fig. 55).

67

ACONITINE IN ACONITE ROOT<sup>21</sup>—TENTATIVE

Crush and macerate the aconite root in a mortar with 15 ml of  $H_2O$  and transfer to extraction tube (Mojonnier type, Fig. 56). Add 5 ml of 10%  $NH_4OH$  and extract 2 or 3 times with 15 ml portions of ether. Transfer ethereal extract to separator and wash with  $H_2O$ . Extract washed, ethereal extract with 2 or 3 ml of 0.02  $N$   $H_2SO_4$ . Test aqueous layer with methyl red indicator; if alkaline, discard aqueous layer.



Continue to extract with 2 or 3 ml of 0.02  $N$   $H_2SO_4$  until the aqueous layer remains acid to methyl red indicator. Test the slightly acid aqueous layer for aconitine by following method:

In small test tube add 1 or 2 drops of 5%  $Na_2CO_3$  to 1 or 2 ml of the slightly acid, aqueous soln. Heat to  $60^\circ$ , stirring with thermometer. Cool, and transfer a few drops of the liquid to microscope slide and examine crystals. Irregular hexagonal plates are formed by aconitine. Most characteristic crystals of aconitine are formed in solns of a strength of 1/1000 or less.

68

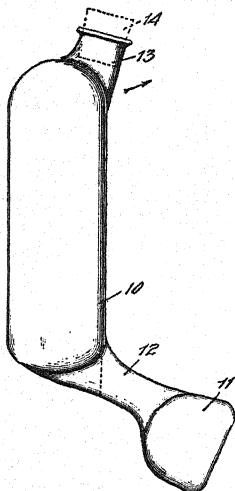
ATROPINE IN TABLETS<sup>22</sup>—OFFICIAL

FIG. 56.—MOJONNIER-TYPE EXTRACTION TUBE

Weigh 25–100 tablets and introduce directly into a small separator. Dissolve in 5–20 ml of  $H_2O$  and add 1 ml of  $NH_4OH$ . Add an equal volume of  $CHCl_3$ , agitate, and allow to stand until separation is complete. Draw off  $CHCl_3$  layer into second separator and repeat extraction with fresh portions of the solvent until the alkaloid is completely removed. After combining all the fractions, wash the combined  $CHCl_3$  solns by agitation with 5 ml of  $H_2O$  and allow to stand 15 min. Introduce pledget of absorbent cotton into stem of separator and carefully draw off  $CHCl_3$  soln into small beaker. Add 10 ml of  $CHCl_3$ , agitate, and when the  $H_2O$  has entirely risen to surface draw off  $CHCl_3$  into beaker. Wash outer surface of stem of separator with a little  $CHCl_3$ , adding washings to beaker. Evaporate soln on steam bath to ca 5 ml. Add a measured excess volume of 0.02  $N$   $H_2SO_4$  and continue evaporation until odor of  $CHCl_3$  has disappeared. Cool soln and titrate back with 0.02  $N$   $NaOH$ , using 1 drop of methyl red indicator. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00578 g of atropine or 0.00695 g of atropine sulfate.

## CINCHONA ALKALOIDS

QUININE, CINCHONIDINE, CINCHONINE, AND QUINIDINE<sup>22</sup>—TENTATIVE

69

## REAGENTS

(a) *Acidified Rochelle salt soln.*—To each 100 ml of a saturated aqueous soln of Rochelle salt add 3 ml of 0.225  $N$   $H_2SO_4$ .

(b) *Washing soln.*—Dilute (a) with an equal volume of  $H_2O$ .

70

## DETERMINATION

Weigh sufficient sample to give ca 0.5 g of total alkaloids, dissolve in 10%  $H_2SO_4$ , filter if necessary, add an excess of  $NH_4OH$ , and extract with  $CHCl_3$  until alkaloids are completely removed. Evaporate combined  $CHCl_3$  extracts on steam bath, add 5 ml of absolute alcohol, and again evaporate; dry at  $110^\circ$  and weigh.

Dissolve residue in 50 ml of 0.225  $N$   $H_2SO_4$ , heat on steam bath 10 min., and make just alkaline (shown by faint permanent precipitate) with a 5% soln of  $NaOH$  added cautiously and with stirring. Add sufficient 0.225  $N$   $H_2SO_4$  to clear soln and then add 5 ml in excess. Add 25 ml of the Rochelle salt soln and stir to start precipitation. Remove from steam bath and place in refrigerator at  $10$ – $15^\circ$ , stirring occasionally for 2 hours. Filter, and wash with 40 ml of the cold washing soln, using small wash bottle and stirring precipitate on filter with rubber-tipped glass rod to remove all soluble alkaloidal salts. Designate combined filtrate and washings as soln "A" and

save for determination of quinidine and cinchonine. Decompose the precipitate of quinine and cinchonidine tartrates with warm 10%  $\text{H}_2\text{SO}_4$  and transfer to a separator, washing filter thoroly to remove all alkaloids. Make alkaline with  $\text{NH}_4\text{OH}$  and remove alkaloids completely by successive extractions with  $\text{CHCl}_3$ . (Four extractions with 25, 20, 15, and 10 ml portions, respectively, are usually sufficient.) Evaporate the combined  $\text{CHCl}_3$  extracts in a weighed beaker containing a little sharp sand, add 5 ml of absolute alcohol, evaporate to dryness on steam bath, heat 3 hours at  $100^\circ$ , cool, and weigh mixed anhydrous alkaloids, quinine and cinchonidine. Add exactly 1 ml of 0.225  $N$   $\text{H}_2\text{SO}_4$  for each 0.015 g of alkaloids and when completely dissolved transfer soln to a polariscope tube, filtering if necessary. Use longest tube possible, reducing its capacity when only small quantities of soln are available by inserting a straight tube of small bore slightly shorter than the polariscope tube and fastened securely as to center. Use a bichromate filter and correct the instrument for operator's eyes. Great precision is necessary for accurate determinations. Read at  $20^\circ$  and calculate to a basis of a 100 mm tube. If the Ventzke scale is used, calculate to angular degrees by multiplying reading by factor 0.34657.

(a) *Quinine*.—Calculate percentage of quinine in the total anhydrous alkaloids obtained in the tartrate separation from following formula, which is based on the specific rotations of quinine ( $-277.4^\circ$ ) and cinchonidine ( $-180^\circ$ ) and the observed rotation of the mixed alkaloids at  $20^\circ$  calculated to a 100 mm tube.

$$Q = (-68.44) (a + 2.7), \text{ in which}$$

$Q$  = % quinine in total anhydrous mixed alkaloids obtained in tartrate separation;  
and

$a$  = observed angular rotation, calculated to 100 mm basis.

(b) *Cinchonidine*.—Determine cinchonidine by difference.

Calculate these results to percentage basis of original sample.

(c) *Quinidine*.—Place soln *A* on steam bath 10 min., add 0.5 g of KI, remove from steam bath, and place in refrigerator at  $10-15^\circ$  for 2 hours, stirring occasionally. Filter on weighed Gooch crucible and wash with 15 ml of ice  $\text{H}_2\text{O}$ , saving filtrate and washings (soln *B*). Dry precipitate of neutral quinidine hydriodide at  $100^\circ$  for 1 hour (a slight yellowing does not affect the results), cool, and weigh. Weight of quinidine hydriodide  $\times 0.717$  = weight of anhydrous quinidine alkaloid.

(d) *Cinchonine*.—Transfer soln *B* to a separator, make alkaline with  $\text{NH}_4\text{OH}$ , and extract completely with  $\text{CHCl}_3$ . Combine  $\text{CHCl}_3$  extracts in weighed beaker containing trace of sharp sand, add 5 ml of absolute alcohol, evaporate to dryness, heat at  $100^\circ$  for 1 hour, cool, and weigh as anhydrous cinchonine alkaloid.

ALKALOID	SPECIFIC ROTATION IN ABSOLUTE ALCOHOL	APPROXIMATE SOLUBILITY IN ETHER	THALLEIOQUIN TEST*
Quinine	laevo $168^\circ$	1 g in 19 ml	Positive
Quinidine	dextro $256^\circ$	1 g in 67 ml	Positive
Cinchonine	dextro $225^\circ$	1 g in 526 ml	Negative
Cinchonidine	laevo $108^\circ$	1 g in 188 ml	Negative

\* *Thalleioquin Test*: Add 1 or 2 drops of Br water to 5 ml of an aqueous soln of the alkaloidal salt (1 in 1000) and 1 ml of  $\text{NH}_4\text{OH}$  (10%). The liquid acquires an emerald-green color due to the formation of thalleioquin.

COCAINE<sup>34</sup>

72

*Method I—Official*

Weigh accurately sufficient uniformly mixed sample to represent 0.1–0.2 g of the alkaloid. Transfer to small separator and dissolve in minimum quantity of  $H_2O$  required for soln. Make soln slightly alkaline with  $NH_4OH$  and extract with successive small portions of peroxide-free ether until alkaloid is completely removed from aqueous soln, using Mayer's reagent, XXXIII, 29(k), for test. Combine ether extracts, remove greater part of the ether by evaporation on steam bath, and allow remainder of ether to evaporate spontaneously at room temp. Dissolve residue in a few ml of neutral alcohol, add 20 ml of 0.05  $N H_2SO_4$ , and titrate the excess of acid with 0.02  $N NaOH$ , using methyl red indicator. 1 ml of 0.05  $N H_2SO_4 = 0.01698$  g of cocaine hydrochloride,  $C_{17}H_{21}O_4N.HCl$ .

73

*Method II—Tentative*

Weigh accurately sufficient uniformly mixed sample to represent ca 0.2 g of the alkaloid. Dissolve in 20 ml of cold  $H_2O$ , add 2 drops of 10%  $HCl$ , and transfer to separator. Make alkaline to litmus with a freshly prepared saturated soln of  $NaHCO_3$  and shake out to exhaustion with petroleum benzin (four 20 ml portions are usually sufficient). Run combined extracts thru plug of absorbent cotton into separator and wash cotton with petroleum benzin. Add a decided excess of 0.02  $N H_2SO_4$ , accurately measured, and shake vigorously several minutes. Separate the 2 layers and wash the petroleum benzin with two 10 ml portions of  $H_2O$ , adding washings to acid soln. Titrate excess acid with 0.02  $N$  alkali, using methyl red indicator, and reserve the titrated soln for the check determination described below. 1 ml of 0.02  $N H_2SO_4 = 0.006793$  g of cocaine hydrochloride,  $C_{17}H_{21}O_4N.HCl$ .

As a check, add 10 ml of 2.5  $N NaOH$  soln to the titrated alkaloidal soln and evaporate on steam bath to ca 10 ml. Cool, transfer soln to separator, and acidify with 10%  $HCl$ . Extract acid soln completely with successive portions of  $CHCl_3$ . Run combined extracts thru plug of absorbent cotton and wash cotton well with  $CHCl_3$ . Allow the  $CHCl_3$  soln to evaporate spontaneously in a weighed beaker, dry residue in vacuum desiccator 2 hours, and weigh. From weight of benzoic acid found calculate its equivalent of cocaine hydrochloride.  $C_6H_5COOH \times 2.782 = C_{17}H_{21}O_4N.HCl$ . (If desired, the benzoic acid may be determined by titration.)

EMETINE HYDROCHLORIDE IN TABLETS<sup>35</sup>—OFFICIAL

74

## PREPARATION OF SAMPLE.—See 1.

75

## DETERMINATION

Transfer to small separator sufficient powdered material, accurately weighed, to represent ca 0.1 g of the alkaloid salt. Dissolve in a minimum quantity of  $H_2O$  and add 5 ml of 4%  $NaOH$  soln. Extract with 30 ml of washed ether, draw off aqueous soln, and swirl separator to remove the  $H_2O$  from the sides. Wash the ether with 1 ml of  $H_2O$ , adding wash  $H_2O$  to aqueous soln. Decant the ether into third separator, washing mouth of separator with ether. Repeat the extractions with 25, 20, 15, and 10 ml portions of ether or until extraction is complete, washing with 1 ml of  $H_2O$  each time, and combine the ether extracts in the third separator. Filter into a beaker thru cotton previously wet with ether, finally wash separator with ether, and evaporate on steam bath, using low temp. to complete evaporation.

To residue add 2 ml of neutral alcohol, cover beaker with watch-glass, and allow to reflux on steam bath for a few minutes. Add a few drops of methyl red indicator, 82(b), and without dilution titrate with 0.02  $N$  acid to faint pink. Cover beaker and

digest on steam bath until all particles are completely dissolved. Cool, and add ca 30 ml of recently boiled  $H_2O$ . Finish titration with standard acid to faint red. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.005533 g of emetine hydrochloride ( $C_{29}H_{40}O_4N_2 \cdot 2HCl$ ).

#### EPHEDRINE IN INHALANTS<sup>86</sup>—OFFICIAL, FIRST ACTION

76

##### DETERMINATION

Weigh accurately into small tared beaker, 5–10 g of sample. Add 10 ml of 2%  $H_2SO_4$ , stir, and allow mixture to stand ca 15 min. Transfer to small separator, rinsing beaker with small portions of ether. Shake gently, and transfer acid layer to a second separator. Shake with 3 successive 10 ml portions of 2%  $H_2SO_4$ , rinsing beaker with ether each time. Test for complete removal of alkaloid.

Neutralize the combined acid soln in separator with  $NH_4OH$ , and add 5 ml in excess. Extract soln with 30 ml of washed ether (automatic extractor optional), transfer aqueous layer to a second separator, and wash ethereal extract with 1 ml of  $H_2O$ , adding washings to main aqueous soln. Swirl the ether in order to remove  $H_2O$  adhering to side of separator. After all  $H_2O$  has been removed, filter mixture into an Erlenmeyer flask thru pledget of cotton wet with ether inserted in a small funnel. Repeat extraction with liberal portions of washed ether at least 4 times, or until the alkaloid is removed completely, washing each portion with the same 1 ml of  $H_2O$ . Evaporate the ether to a volume of 10 ml by aid of current of air. Add bromothymol blue indicator, VI, 117(e), a measured excess of 0.02  $N$   $H_2SO_4$  and ca 40 ml of  $CO_2$ -free  $H_2O$ ; cover with watch-glass, heat on steam bath in order to dissolve the alkaloid adhering to sides of flask and evaporate all the ether. Cool, and titrate excess acid with 0.02  $N$   $NaOH$ , using indicator standard, pH 6.0, for comparison. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.0033 g of ephedrine.

#### EPHEDRINE IN TABLETS<sup>87</sup>—OFFICIAL, FIRST ACTION

77

##### PREPARATION OF SAMPLE.—See 1.

78

##### DETERMINATION

Powder in mortar not less than 20 tablets, weigh accurately a quantity equal to ca 0.12 g of the alkaloidal salt, and transfer to a separator. Dissolve in minimum quantity of  $H_2O$ , then add 5 ml of  $NH_4OH$ . Extract the soln with 30 ml of washed ether. Transfer aqueous layer to a second separator. Wash the ether extract with 1 ml of  $H_2O$ , adding washings to main aqueous soln. Swirl the ether in order to remove  $H_2O$  adhering to side of separator. After all  $H_2O$  has been removed, filter into a beaker thru pledget of cotton wet with ether inserted in small funnel. Repeat extraction with liberal portions of ether at least 4 times, or until the alkaloid is removed completely, washing each portion with 1 ml of  $H_2O$ . Evaporate ether to volume of 10 ml on steam bath without heat and proceed as directed in 76, beginning "Add bromothymol blue." 1 ml of 0.02  $N$  acid = 0.00403 g of ephedrine hydrochloride, 0.00428 g of ephedrine sulfate, and 0.0033 g of ephedrine.

#### ALKALOIDS IN ERGOT<sup>88</sup>—TENTATIVE

(Applicable to ergotamine and ergotoxine.)

79

##### REAGENTS

(a) *Dimethylaminobenzaldehyde soln.*—Add 650 ml of  $H_2SO_4$  to ca 300 ml of  $H_2O$ . Cool, add 1.25 g of paradimethylaminobenzaldehyde, 0.05 g of  $FeCl_3 \cdot 6H_2O$ , and sufficient  $H_2O$  to make 1 liter.

(b) *Ergotoxine ethanesulfonate standard soln.*—With the aid of an excess of tartaric acid, dissolve sufficient ergotoxine ethanesulfonate, accurately weighed, gradually

adding  $\text{H}_2\text{O}$ , to yield a soln containing 0.01 g of ergotoxine ethanesulfonate and 0.05 g of tartaric acid in 100 ml.

(c) *Ergotamine tartrate standard soln.*—Prepare as directed in (b), using ergotamine tartrate instead of ergotoxine ethanesulfonate.

80

## EXTRACTION OF ALKALOIDS

Pipet 5 ml of the fluidextract at  $20^\circ$  into a separator and dilute with 30 ml of  $\text{H}_2\text{O}$ . Add 2 ml of  $\text{NH}_4\text{OH}$  (1+10) or sufficient to make distinctly alkaline to litmus paper. Extract with peroxide-free ether, using 40, 25, 20, 15 ml portions, or until the alkaloids are removed completely. To assure complete extraction make an additional extraction with 20 ml of ether, evaporate in a separate beaker, dissolve residue in 1 ml of 1% tartaric acid soln, and test with reagent for blue color. Combine the ether extracts in separator, wash at least 3 times with 25 ml portions of  $\text{H}_2\text{O}$  containing 3 drops of  $\text{NH}_4\text{OH}$  to remove yellow pigments, and finally wash twice with  $\text{H}_2\text{O}$  to remove excess of alkali. Transfer combined washings to a separator, extract with three 5 ml portions of ether, wash combined portions with  $\text{H}_2\text{O}$ , and add to main ether extracts. Shake ether with aqueous 1% tartaric acid soln, using 10, 10, 10, and 5 ml portions, respectively, until alkaloids are removed completely. Evaporate combined acid solns on water bath in current of air to remove ether, transfer soln to a 25 ml volumetric flask, and make to volume.

81

## COLORIMETRIC COMPARISONS

Pipet 1 ml of the standard soln at  $20^\circ$  into glass colorimeter cup or test tube and add 2 ml of the reagent. Mix. Pipet 1 ml of the extracted ergot soln to a second cup or test tube and add 2 ml of the reagent. Mix. Allow to stand 30 min., or until blue color reaches maximum intensity. Read in colorimeter. Repeat comparison if necessary with aliquots of the alkaloidal solns to produce about same color intensity as standard. Calculate percentage of total alkaloids of ergot as ergotamine tartrate or ergotoxine ethanesulfonate.

HOMATROPINE IN TABLETS<sup>22</sup>—OFFICIAL

82

## REAGENTS

(a) *Iodine soln.*—See 5(b).

(b) *Methyl red indicator.*—Dissolve 0.1 g of methyl red in 100 ml of neutralized alcohol and filter if necessary.

83

## PREPARATION OF SAMPLE.—See 1.

84

## DETERMINATION

Weigh accurately a quantity of sample equal to ca 0.130 g of the alkaloidal salt, and transfer to separator. Dissolve in 10–20 ml of  $\text{H}_2\text{O}$  and add 2 ml of  $\text{NH}_4\text{OH}$ . Add ca 20 ml of  $\text{CHCl}_3$ , agitate, and allow to stand until separation is complete. Draw off  $\text{CHCl}_3$  layer into second separator and repeat extraction with fresh portions of the solvent until alkaloid is completely removed (5 extractions usually suffice). Test for complete removal with the I reagent. After combining all the fractions, wash  $\text{CHCl}_3$  solns by agitation with 5 ml of  $\text{H}_2\text{O}$  and allow to settle. Filter the  $\text{CHCl}_3$  soln thru cotton into a small beaker. Wash aqueous soln with 10 ml of  $\text{CHCl}_3$ ; draw off the  $\text{CHCl}_3$  and filter into the beaker. Wash outer surface of stem of separator and the funnel and its stem with a little  $\text{CHCl}_3$ , adding washings to beaker. Evaporate the soln on steam bath to ca 5 ml. Add a measured excess of 0.02 N  $\text{H}_2\text{SO}_4$ . Place beaker in warm place and evaporate with aid of fan until odor of  $\text{CHCl}_3$  has disappeared. Cool the soln and titrate back with 0.02 N NaOH, using 1 drop of methyl red indicator. 1 ml of 0.02 N  $\text{H}_2\text{SO}_4$  = 0.007122 g of homatropine hydrobromide or 0.006233 g of homatropine hydrochloride.

## OPIUM ALKALOIDS AND THEIR DERIVATIVES

85

APOMORPHINE IN TABLETS<sup>10</sup>—OFFICIAL

Weigh a number of tablets equivalent to ca 0.065 g (1 grain) of the alkaloid or of its salt and dissolve in 10 ml of  $H_2O$  in separator. Add 1 ml of freshly prepared saturated soln of  $NaHCO_3$  and 25 ml of peroxide-free ether, and shake mixture. After separation, draw off lower layer into a second separator and transfer ethereal layer to a third separator. Extract mixture in second separator repeatedly with 15 ml portions of ether until alkaloid has been completely removed, using second and first separators alternately for the shaking, and collecting all ethereal soln in the third. Discard aqueous soln. Wash ethereal soln of alkaloid 3 times with 5 ml portions of  $H_2O$ , uniting aqueous washings in a clean separator. Extract these washings with a little fresh peroxide-free ether. Discard aqueous portion, wash ether with  $H_2O$ , discard washings, and add washed ether to main portion of ethereal soln. Add 20 ml of 0.02  $N$   $H_2SO_4$  to ethereal soln of alkaloid in separator and shake mixture thoroly. Transfer mixture to a beaker, wash separator twice with 5 ml portions of  $H_2O$ , adding washings to acid liquid in beaker, and without delay evaporate ether at low temp., preferably on water bath with aid of blast of air. Titrate excess acid with 0.02  $N$   $NaOH$ , using one drop of methyl red indicator, 82(b). 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00625 g of apomorphine hydrochloride,  $C_{17}H_{17}O_2N \cdot HCl \cdot \frac{1}{2}H_2O$ .

CODEINE IN TABLETS<sup>11</sup>

86

*Qualitative Tests—Official*

(a) To the residue or tablet add  $HNO_3$ . A yellow color is produced.

(b) To 3 ml of an aqueous soln (1+200), add a few drops of a 10%  $K_3Fe(CN)_6$  soln and 1 or 2 drops of 10%  $FeCl_3 \cdot 6H_2O$  soln. A green color is produced.

87

*Quantitative Method—Official*

Transfer to a small separator sufficient tablets, or powdered material equal to a multiple of average weight per tablet, to represent ca 0.15 g of the alkaloid. Dissolve in minimum quantity of  $H_2O$  (not over 5 ml) acidified with 2 drops of  $HCl$ . Add solid  $NaHCO_3$  until neutralized, then a slight excess, and extract 5 times with  $CHCl_3$ , using 30, 20, 20, 10, and 5 ml. Test for complete extraction of the alkaloid. (Make an additional extraction with 10 ml of  $CHCl_3$ , evaporate solvent in separate beaker, and dissolve residue in a few drops of methyl alcohol. Add a drop of methyl red indicator, 82(b), and dilute with 20 ml of  $H_2O$ , carbonate free. A yellow color indicates incomplete extraction. Titrate, and add quantity thus obtained to total.) Combine  $CHCl_3$  extracts in a second separator, into stem of which is inserted pledget of cotton wet with  $CHCl_3$ . Wash combined extracts with 1 ml of  $H_2O$  containing 1 drop of  $NH_4OH$  and proceed as directed under 93, beginning "Evaporate on a water bath." 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00787 g of codeine sulfate,  $(C_{18}H_{21}O_3N)_2 \cdot H_2SO_4 \cdot 5H_2O$ , or 0.00849 g of codeine phosphate,  $C_{18}H_{21}O_3N \cdot H_3PO_4 \cdot 1\frac{1}{2}H_2O$ .

DIACETYLMORPHINE (HEROIN) IN TABLETS<sup>12</sup>

88

*Qualitative Test—Official*

Heat ca 0.1 g with 1 ml of  $H_2SO_4$  and 1 ml of alcohol. Ethyl acetate, readily recognized by its odor, is formed.

89

*Quantitative Method—Official*

Weigh, and transfer directly to small separator a number of tablets representing ca 0.15 g of diacetylmorphine. Dissolve in 5 ml of  $H_2O$  containing 1 drop of acetic

acid. Add 1 ml of  $\text{NH}_4\text{OH}$  and extract 5 times with  $\text{CHCl}_3$ , using 30, 20, 10, 10, and 5 ml, respectively. Combine  $\text{CHCl}_3$  extracts in a second separator, into stem of which is inserted pledget of cotton wet with  $\text{CHCl}_3$ . Wash combined extracts with 1 ml of  $\text{H}_2\text{O}$  and proceed as directed under 65, beginning "Evaporate on water bath." 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.008473$  g of diacetylmorphine hydrochloride,  $\text{C}_{21}\text{H}_{23}\text{O}_5\text{N} \cdot \text{HCl} \cdot \text{H}_2\text{O}$ .

#### MORPHINE IN TABLETS<sup>43</sup>

90

##### *Qualitative Tests—Official*

(a) To the residue or tablet add  $\text{HNO}_3$ . An orange-red color fading to yellow is produced.

(b) To an aqueous soln add a few drops of a 10%  $\text{K}_3\text{Fe}(\text{CN})_6$  soln and then a drop of a 10%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  soln. A deep blue soln results; a blue precipitate separates on standing.

(c) See Microchemical Tests, 222.

##### *Quantitative Method—Official*

91

#### REAGENT

*Alkaline salt soln.*—Dissolve 30 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , dilute to 1 liter, add  $\text{NaCl}$  to saturation, and filter.

92

#### PREPARATION OF SAMPLE

To ascertain variation in weight, weigh separately at least 20 tablets. Also weigh collectively a representative number of unbroken tablets and calculate average weight per tablet. To insure representative sampling in tablets containing more than  $\frac{1}{4}$  grain of alkaloid, pulverize ca 20 tablets, mix powder thoroly, and protect from moisture in well-stoppered bottle.

93

#### DETERMINATION

Transfer to a small separator sufficient tablets, or powdered material equal to a multiple of average weight per tablet, to represent ca 0.15 g of the alkaloid. Moisten with 5 ml of  $\text{H}_2\text{O}$ , shake gently, and then dissolve completely by adding 10 ml of the alkaline salt soln. (Excipients may not be completely soluble.) To the alkaline salt soln, add a small piece of litmus paper and then  $\text{HCl}$ , dropwise, until it is neutral. Add 10 drops in excess. Add 5 ml of alcohol, carefully neutralize with  $\text{NH}_4\text{OH}$  dropwise, and then add 5 drops in excess. Invert separator and open stopcock to insure neutralization of residual acid. Immediately extract, at least 6 times, with  $\text{CHCl}_3$ -alcohol solvent (90+10), using 30, 20, 20, 10, 10, and 5 ml, or until the alkaloid is completely removed. Test for complete extraction of alkaloid. (Make an additional extraction with 10 ml of the  $\text{CHCl}_3$ -alcohol solvent, evaporate solvent in a separate beaker, dissolve residue in a few drops of methyl alcohol, add a drop of methyl red, 82(b), and dilute with 20 ml of  $\text{H}_2\text{O}$ , carbonate free. A yellow color indicates incomplete extraction. Titrate, and add quantity thus obtained to total.) Combine  $\text{CHCl}_3$ -alcohol extracts in a second separator, into stem of which is inserted pledget of cotton wet with  $\text{CHCl}_3$ . Wash combined extracts with 1 ml of  $\text{H}_2\text{O}$ . When clear, filter into a small beaker. Extract the wash  $\text{H}_2\text{O}$  twice with small portions of the  $\text{CHCl}_3$ -alcohol solvent. Evaporate on water bath, using electric fan to prevent decrepitation of the residue. When dry, remove immediately and complete determination by one of following procedures:

(a) To the alkaloidal residue add 2–3 ml of methyl alcohol, cover beaker with watch-glass, and heat on steam bath until residue, including any portions thereof that may adhere to upper part of beaker, is completely dissolved. Add 2 drops of

the methyl red indicator and, without dilution with  $H_2O$ , titrate carefully with 0.02  $N$   $H_2SO_4$  to faint pink, avoiding excess. Cover beaker and digest on steam bath until all particles are completely dissolved. If more than 2 ml of alcohol is added, evaporate excess. Cool, and dilute with 50 ml of boiled  $H_2O$ . (Soln should now be yellow.) Finish titration with the standard acid to faint red.

(b) Dissolve residue in 2–3 ml of methyl alcohol on steam bath. Add 2 drops of the methyl red indicator and then add from buret 5–10 ml excess of 0.02  $N$   $H_2SO_4$ , noting total quantity used. Cover beaker with watch-glass and heat on steam bath until residue, including any portions thereof that may adhere to upper part of beaker, is completely dissolved. Dilute with 50 ml of cold, previously boiled  $H_2O$ . Titrate back with the 0.02  $N$   $NaOH$  soln. The  $H_2O$  and alkali should be sufficiently free from carbonates to insure a sharp end point with methyl red. 1 ml of 0.02  $N$  acid = 0.007513 g of morphine hydrochloride,  $C_{17}H_{19}O_3N \cdot HCl \cdot 3H_2O$ , or 0.007585 g of morphine sulfate,  $(C_{17}H_{19}O_3 \cdot N)_2 \cdot H_2SO_4 \cdot 5H_2O$ .

Alkaloids other than morphine are extracted by  $CHCl_3$ , while morphine remains in the fixed alkali soln. In general, this separation is unnecessary. If the tablets are of unknown composition or atropine or scopolamine is present, shake the alkaline salt soln with 10 ml portions of washed  $CHCl_3$  (use ether for separation of atropine). Transfer clear solvent to a small beaker and evaporate on steam bath. If a residue is obtained, apply usual tests.

## 94

MORPHINE IN SIRUPS<sup>44</sup>—OFFICIAL

Shake bottle well and transfer 50 ml to a 150 ml pear-shaped separator. Add a few drops of 10% ammonia water to insure a weak alkaline reaction and test with litmus paper. Extract total alkaloids with mixture of  $CHCl_3$  and alcohol (9+1). (About seven 25 ml portions are necessary, depending on care of separating solvent and length and violence of each shake-out. Larger quantities of solvent may be used to insure absence of emulsions.) Combine solvents and wash with 5 ml of  $H_2O$ . Run thru  $CHCl_3$ -wetted cotton. Evaporate solvent. (If the sirup is known to carry pure morphine alkaloid or its salt, and no other alkaloid, this residue may be dissolved in alcohol and filtered, and the soln titrated.) Dissolve in 2 ml of 5%  $HCl$  on water bath, covering beaker to insure complete soln. Add 20 ml of  $H_2O$  and transfer to a separator. Make alkaline with 5 ml of 5%  $KOH$  soln and exhaust with three 20 ml portions of  $CHCl_3$  followed by two 20 ml portions of petroleum benzin (removal of non-phenolic alkaloids and  $CHCl_3$ ). Combine immiscible solvents and wash with 5 ml of  $H_2O$ . Discard solvent and add wash water to main aqueous soln.

Render the aqueous soln acid with 5%  $HCl$  and then just alkaline with a few drops of 10% ammonia and extract with three 20 ml portions of petroleum benzin for removal of petroleum benzin-soluble phenolic alkaloids. Wash combined petroleum benzin with 5 ml of  $H_2O$ . Discard the petroleum benzin and add the wash water to main aqueous soln. Saturate with  $NaCl$ .

Extract the morphine completely with seven 25 ml portions of the  $CHCl_3$ -alcohol mixture. Combine portions of solvent. Wash with 5 ml of  $H_2O$  and run solvent thru plug of  $CHCl_3$ -saturated cotton. Evaporate the solvent. Dissolve residue in 5 ml of neutralized alcohol in a covered beaker by aid of heat on steam bath. Add excess of 0.02  $N$   $H_2SO_4$  and titrate back with 0.02  $N$  alkali, using methyl red indicator. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.0076 g of morphine sulfate,  $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ .

## 95

Pilocarpine Hydrochloride in Tablets<sup>45</sup>—OFFICIAL, FIRST ACTION

Ascertain average weight per tablet. Pulverize, mix thoroly, and weigh out a sufficient portion to represent 1 grain of the salt. Dissolve sample in 10 ml of  $H_2O$ ,



add 1 ml of 10%  $\text{NH}_4\text{OH}$ , and shake out rapidly with 20 ml of  $\text{CHCl}_3$ . Repeat extraction, using 15 ml of  $\text{CHCl}_3$ , and complete with successive 10 ml portions. Filter each portion of  $\text{CHCl}_3$  thru pledget of cotton and combine in a 250 ml beaker, finally washing stem of separator and funnel with  $\text{CHCl}_3$ . Evaporate on steam bath until the  $\text{CHCl}_3$  soln measures ca 5 ml. Add 20 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$  and evaporate remainder of  $\text{CHCl}_3$ . Titrate excess acid with 0.02  $N$   $\text{NaOH}$ , using 1 drop of methyl red as indicator. (End point is not particularly sharp, but with care it can be obtained.) 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.004893$  g of pilocarpine hydrochloride,  $\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2 \cdot \text{HCl}$ .

#### PROCAINE

96

##### *Qualitative Tests<sup>48</sup>—Official*

(a) Dissolve 0.1 g of sample in ca 10 ml of  $\text{H}_2\text{O}$ . Add 2 ml of 5%  $\text{KMnO}_4$  soln. Warm, if necessary. Reduction occurs with evolution of gas having odor of acetaldehyde (distinction from cocaine, which does not readily reduce  $\text{KMnO}_4$ ).

(b) Dissolve ca 0.005 g of sample in 3 ml of  $\text{H}_2\text{O}$  and add a few drops of Mayer's reagent, XXXIII, 29(k). With procaine a white precipitate is formed, which dissolves after addition of a few ml of  $\text{H}_2\text{SO}_4$  (1+49). (Precipitates with stovaine and cocaine are not readily soluble in dilute  $\text{H}_2\text{SO}_4$ .)

(c) Dissolve ca 0.1 g of procaine in 2 ml of  $\text{H}_2\text{O}$ . From a buret add 25 ml of 0.1  $N$   $\text{NaOH}$ . (White precipitate formed dissolves in excess of the  $\text{NaOH}$  when heated.) Heat soln 25 min. on steam bath. Upon cooling the soln, extracting with  $\text{CHCl}_3$ , and evaporating solvent, no residue should be obtained. (Stovaine does not readily hydrolyze, and a residue giving an alkaloidal reaction remains upon evaporation of the  $\text{CHCl}_3$ .)

##### *Quantitative Methods*

97

##### *Method I—Official*

(Determines as procaine any *p*-amino-benzoic acid due to decomposition of procaine.)

Dissolve quantity of sample equivalent to ca 0.1 g of procaine hydrochloride in 5 ml of  $\text{H}_2\text{O}$  in a 50 ml beaker. Add 25 ml of 0.1  $N$   $\text{NaOH}$  and heat on steam bath 25 min. Cool, and transfer soln to a 500 ml glass-stoppered flask. Add 50 ml of standard bromide-bromate soln, 26(c), dilute with  $\text{H}_2\text{O}$  to 250 ml, add 10 ml of  $\text{HCl}$ , and stopper flask immediately to avoid loss of  $\text{Br}$ . Shake flask occasionally and allow to stand 30 min. at room temp., keeping the flask tightly stoppered. (It is necessary that a large excess of  $\text{Br}$  be present, as shown by a bright yellow color.) Add quickly 10 ml of 20%  $\text{KI}$  soln, stopper, and shake flask. Allow to stand 15 min., shaking occasionally. Titrate excess  $\text{I}$  with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  soln, using starch indicator, VI, 3(e). Titrate to disappearance of the blue color, disregarding color that reappears on standing. 1 ml of 0.1  $N$  bromide-bromate soln = 0.00454 g of procaine hydrochloride,  $\text{C}_{13}\text{H}_{20}\text{O}_2\text{N}_2 \cdot \text{HCl}$ .

98

##### *Method II—Official*

(Determines only undecomposed procaine.)

Weigh quantity of powder or number of tablets equivalent to ca 0.2 g of procaine. Dissolve in 10–15 ml of  $\text{H}_2\text{O}$ , transfer soln to a separator, and add ca 3 ml of  $\text{NH}_4\text{OH}$ . Extract the ammoniacal soln 4 or 5 times with  $\text{CHCl}_3$ , using 15 ml for first extraction and 10 ml for subsequent extractions. Filter into a weighed beaker and evaporate the  $\text{CHCl}_3$  by means of electric fan, preferably at room temp., avoiding prolonged heating of procaine base, as it is slightly volatile at  $100^\circ$ . Take up residue

with a slight excess of 0.1 *N* or 0.02 *N* H<sub>2</sub>SO<sub>4</sub>. Titrate excess of acid with 0.02 *N* NaOH, using methyl red indicator. 1 ml of 0.1 *N* H<sub>2</sub>SO<sub>4</sub> = 0.02726 g of C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>N<sub>2</sub>·HCl. 1 ml of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> = 0.00545 g of C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>N<sub>2</sub>·HCl. C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>N<sub>2</sub> × 1.1544 = procaine hydrochloride (novocaine).

99

*Method III*<sup>47</sup>—*Tentative*

(Applicable in presence of chlorobutanol, cocaine, codeine, heroine, lactose, and morphine.)

Weigh into Kjeldahl flask 0.3–0.5 g of procaine or one of its salts, or measure an equivalent of an ampul soln. Dissolve in 150 ml of H<sub>2</sub>O (or add sufficient H<sub>2</sub>O to make 150 ml), and add 2 ml of 50% NaOH. Quickly connect to condenser and distil 100 ml into flask containing measured excess of standard acid, extending delivery tube below surface of the soln. Remove receiver, rinse condenser with a little H<sub>2</sub>O, and titrate excess acid with standard alkali, using methyl red as indicator. Each ml of 0.1 *N* acid consumed = 0.0236 g of procaine, C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>N<sub>2</sub>, or 0.0273 g of procaine hydrochloride, C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>N<sub>2</sub>·HCl.

100

STRYCHNINE IN TABLETS<sup>48</sup>—OFFICIAL

(Other alkaloids absent.)

Count and weigh sufficient tablets (or pills) to represent 1 grain of the alkaloidal salt and transfer to small beaker. If the color on coated tablets interferes with the indicator in titration, wash without removing the strychnine. Add 10 ml of 5% HCl, disintegrate tablets with stirring rod, warm on steam bath ca 10 min., cool, and transfer to separator with not more than 10 ml of H<sub>2</sub>O. To remove all the strychnine, add to beaker 2 ml of NH<sub>4</sub>OH (or an excess) and 25 ml of CHCl<sub>3</sub>, rinse, and add to separator. Then rinse beaker with portions of CHCl<sub>3</sub> to be used for each extraction. Extract 5 times with CHCl<sub>3</sub>, using 25, 20, 15, 10, and 5 ml portions, or until alkaloid is completely removed. Combine the first two extractions in a second separator, in stem of which is a pledget of absorbent cotton wet with CHCl<sub>3</sub>. Wash with 5 ml of H<sub>2</sub>O containing a drop of NH<sub>4</sub>OH (1+2). When clear, filter the CHCl<sub>3</sub> portion in small beaker. Wash each successive CHCl<sub>3</sub> extract with the same wash water and filter in a similar manner into the main portion, finally washing outer surface of stem of separator with a few ml of CHCl<sub>3</sub> and adding this also to main portion. Evaporate on steam bath, removing dish from bath as last portions evaporate to avoid decrepitation.

Add 2–5 ml of neutral alcohol, cover beaker, and warm on steam bath to dissolve residue. If necessary, add just enough additional neutral alcohol to complete the soln. Add 2 drops of methyl red indicator, and titrate with 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to faint pink color. If more than 2 ml of alcohol was used, evaporate excess, cool, dilute with 50 ml of recently boiled H<sub>2</sub>O, and continue titration with the 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to faint pink color. If preferred, add an excess of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to the alcoholic soln of the alkaloids, evaporate the alcohol if necessary as directed above, and titrate excess acid with 0.02 *N* NaOH.

1 ml of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> = 0.006684 g of C<sub>21</sub>H<sub>22</sub>O<sub>2</sub>N<sub>2</sub>, 0.008565 g of (C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>)<sub>2</sub>·H<sub>2</sub>SO<sub>4</sub>·5H<sub>2</sub>O, or 0.007944 g of C<sub>21</sub>H<sub>22</sub>O<sub>2</sub>N<sub>2</sub>·HNO<sub>3</sub>.

101

STRYCHNINE IN LIQUID PREPARATIONS<sup>49</sup>—OFFICIAL

(Other alkaloids absent.)

Measure into evaporating dish 50 ml of sample, or a quantity sufficient to yield at least 0.065 g of the alkaloid, and remove the alcohol by evaporation. Transfer

to separator, add 1 ml of  $\text{NH}_4\text{OH}$ , or sufficient to render the soln alkaline, and proceed as directed under 100, beginning "Extract 5 times with  $\text{CHCl}_3$ ."

#### SEPARATION OF QUININE AND STRYCHNINE<sup>50</sup>—TENTATIVE

102

##### REAGENT

*Bromocresol purple soln.*—Triturate 0.100 g of bromocresol purple in agate mortar with 9 ml of 0.02  $N$   $\text{NaOH}$ . After soln dilute with  $\text{H}_2\text{O}$  to 200 ml, and filter if necessary. The soln should be deep orange to red in color. If it is purple, the addition of not more than 0.5 ml of 0.02  $N$  acid should make it red. If it is yellow, the addition of not more than 0.5 ml of 0.02  $N$  alkali should produce the red color.

103

##### TOTAL ALKALOIDS

Make 50 ml of the soln acid with citric acid, add an equal volume of  $\text{H}_2\text{O}$ , evaporate to nearly the original volume to remove excess alcohol, cool, and extract with two 15 ml portions of ether to remove oily material. Make aqueous soln alkaline with  $\text{NH}_4\text{OH}$  and extract mixed alkaloids in usual way with mixture of 2 parts of  $\text{CHCl}_3$  and 1 part of ether, using 25, 20, 15, 10, and 5 ml portions. Evaporate the  $\text{CHCl}_3$  and ether in a weighed Erlenmeyer flask or beaker to dryness on steam bath. Add a little ether and again evaporate to dryness to remove the last traces of  $\text{CHCl}_3$ . Dry at  $100^\circ$  for 1 hour and weigh to obtain approximate weight of mixed alkaloids.

(a) *Strychnine*.—Dissolve alkaloidal residue in 50 ml of 10%  $\text{H}_2\text{SO}_4$ , add 5 ml of 4%  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  dropwise from buret, stirring well, and set aside a few hours, or overnight. Collect resulting precipitate on small (7 cm) filter and wash 3 times with 3 ml of 5%  $\text{H}_2\text{SO}_4$ . Reserve filtrate for determination of quinine. Wash precipitate immediately into a small separator with  $\text{H}_2\text{O}$ , transferring precipitate remaining in flask to separator by shaking about 3 times with 3 ml of  $\text{NH}_4\text{OH}$  and a small quantity of  $\text{CHCl}_3$ . Extract the ammoniacal soln of the precipitate with 25, 15, 15, 10, and 5 ml portions of  $\text{CHCl}_3$ . Collect the  $\text{CHCl}_3$  solns in another separator and extract the alkaloids by shaking with 25, 10, 10, and 5 ml portions of 20%  $\text{H}_2\text{SO}_4$ ; repeat precipitation with  $\text{K}_4\text{Fe}(\text{CN})_6$  and the other operations until the  $\text{CHCl}_3$  extracts are again obtained, reserving filtrate for determination of quinine. Evaporate the  $\text{CHCl}_3$  carefully, adding a little alcohol toward end to prevent spattering. Weigh residue of strychnine after drying it for 1 hour at  $100^\circ$  (should be nearly white and free from quinine). Check volumetrically as follows: Dissolve residue in hot alcohol, add 0.02  $N$   $\text{H}_2\text{SO}_4$  until soln is acid to methyl red indicator, 82(b), then add 2 or 3 ml in excess. Evaporate most of the alcohol, cool, and titrate back with 0.02  $N$  alkali. 1 ml of 0.02  $N$  acid = 0.006684 g of strychnine,  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2$ , or 0.008565 g of strychnine sulfate,  $(\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2)_2 \cdot \text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ .

(b) *Quinine*.—Combine the 2 filtrates from the precipitations with  $\text{K}_4\text{Fe}(\text{CN})_6$  in separator, make alkaline with  $\text{NH}_4\text{OH}$ , and extract with mixture of 2 parts of  $\text{CHCl}_3$  and 1 part of ether, using 20, 15, 15, 10, and 5 ml portions of the solvent and observing the usual precaution of washing stem of separator with the  $\text{CHCl}_3$ -ether mixture. Wash combined extractions in a second separator with two 5 ml portions of  $\text{H}_2\text{O}$ , transfer to weighed beaker, evaporate to dryness, add a few ml of ether, and again evaporate to dryness to remove final traces of  $\text{CHCl}_3$ . Dry at  $120$ – $130^\circ$ , cool, and weigh as anhydrous quinine. Test residue qualitatively for quinine, or if desired, check the quantity volumetrically as follows: Dissolve residue in a little alcohol, add 7 drops of the bromocresol purple indicator, then add 0.02  $N$   $\text{H}_2\text{SO}_4$  to a yellow color, and 1 ml in excess. Evaporate the soln to small volume, cool,

allow the quinine sulfate to separate, filter thru small pledget of cotton in stem of a funnel, wash with small portions of  $H_2O$ , and titrate combined filtrate and washings with 0.02 *N* alkali. 1 ml of 0.02 *N*  $H_2SO_4 = 0.006484$  g of anhydrous quinine,  $C_{20}H_{24}O_2N_2$ ; 0.007565 g of quinine alkaloid,  $C_{20}H_{24}O_2N_2 \cdot 3H_2O$ ; or 0.007825 g of quinine sulfate,  $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ .

104

THEOBROMINE IN THEOBROMINE CALCIUM<sup>51</sup>*Method I—Tentative*

Dry ca 0.5 g of the material at 110° to constant weight. Weigh 0.2 g of the dried substance into a glass-stoppered 100 ml volumetric flask, add 2 ml of glacial acetic acid, and warm on steam bath. Add 10 ml of boiling  $H_2O$  and shake until soln has taken place, adding more boiling  $H_2O$  if necessary. Cool soln to room temp. (Soln should be clear or nearly so.) Add 50 ml of 0.1 *N* iodine, 20 ml of saturated salt soln, and 2 ml of  $HCl$ . Shake well and make to volume with  $H_2O$ . Shake again and allow to stand overnight. Filter, discarding first 10 ml of filtrate. Titrate 50 ml of the filtrate with 0.1 *N*  $Na_2S_2O_3$ , using starch soln, VI, 3(e), as indicator. 1 ml of 0.1 *N*  $I = 0.0045$  g of theobromine,  $C_7H_8O_2N_4$ .

*Method II—In Tablets<sup>52</sup>—Tentative*

105

## INDICATOR

*Phenol red soln.*—Triturate 0.1 g of phenol red in an agate mortar with 15 ml of 0.02 *N*  $NaOH$  until dissolved and dilute the soln with recently boiled  $H_2O$  to 200 ml.

106

## DETERMINATION

Place 0.5 g of the powdered tablets, previously dried at 110°, or 0.4 g of theocalcin powder, or 0.2 g of theobromine alkaloid in a 300 ml beaker and add 100 ml of  $H_2O$ . Warm moderately over a flame and add 15 ml of approximately 0.1 *N*  $H_2SO_4$ . Heat to boiling to insure complete soln and to remove  $CO_2$ . Cool to room temp. Add 1.5 ml of the phenol red indicator and render slightly alkaline with ca 0.1 *N*  $NaOH$  (yellow color). To this soln add 25 ml (an excess) of neutral 0.1 *N*  $AgNO_3$ , VI, 89, and titrate the liberated  $HNO_3$  immediately with 0.1 *N*  $NaOH$  to a distinctly violet red color. Titrate cautiously dropwise with constant stirring near end point. 1 ml of 0.1 *N*  $NaOH = 0.018$  g of  $C_7H_8O_2N_4$ .

107

THEOPHYLLINE<sup>53</sup>—TENTATIVE

(Applicable to solutions and tablets.)

Weigh 0.2–0.3 g of theophylline (or an equivalent quantity of soln or powdered tablets) into a separator. Add 5 ml of 0.5 *N*  $NaOH$  and shake mixture gently until alkaloid is dissolved. Add a strip of litmus paper and sufficient 0.5 *N*  $HCl$  from buret to produce a distinct acid reaction. Then add 0.5 ml more of the acid. Add 30 ml of  $CHCl_3$ -isopropyl alcohol mixture (3+1) and shake 1 min. Allow to settle and draw off lower layer into a second separator that contains 10 ml of  $H_2O$  acidified with  $HCl$ . Shake well, allow to settle, and filter solvent into a weighed flask thru pledget of cotton placed in stem of a funnel. Repeat extraction with 6 more portions of 20 ml each of the  $CHCl_3$ -isopropyl alcohol mixture, wash each portion thru the second separator, and pass solvent thru filter into the weighed flask. Insure complete extraction by a seventh shaking with 10 ml of the solvent and evaporation of the washed solvent in a separate container. Recover most of solvent and evaporate

remainder on steam bath while rotating container in an inclined position. Add 2 ml of absolute ether to residue and evaporate (cautiously to avoid spattering). Dry residue at  $80^{\circ}$  to constant weight and weigh as anhydrous theophylline.  $C_7H_8O_2N_4 \times 1.10 = C_7H_8O_2N_4 \cdot H_2O$ .

## 108

ALOIN<sup>54</sup>—OFFICIAL

(Applicable to mixtures containing cascara, rhubarb, senna, and other acid hydrolyzable anthraglucosides, as well as to resins and phenolphthalein with aloin or aloes.)

Dry sufficient powdered material 1 hour at  $110^{\circ}$  (or dealcoholized soln if a liquid) to insure ca 0.3 g of aloin. Add 10 ml of  $H_2O$  and a few ml of 5% NaOH soln. Transfer

mixture to a 100 ml volumetric flask, dilute to ca 75 ml, and make acid with  $H_2SO_4$ , working rapidly as aloin is attacked by alkali. Dilute to mark, and add a few glass beads if much undissolved material is present. Shake occasionally during an hour to insure soln of aloin. Filter, and transfer a 40 ml aliquot, to which has been added 10 ml of 10%  $H_2SO_4$  (by weight), to a continuous extraction apparatus previously charged with  $CHCl_3$  (A, Fig. 57). Reflux to exhaustion (ca 2 hours). Disconnect apparatus and transfer all aqueous soln to a separator, discarding the  $CHCl_3$ . Saturate soln with salt and shake out with 30 ml portions of  $CHCl_3$ -alcohol mixture (3+1). Test for complete removal of aloin by evaporating a portion of the 6th extraction (more extractions may be necessary). Shake violently. Combine extracts and wash with 1 ml of  $H_2O$ , to which is added 1 g of  $NaHCO_3$ , or more if necessary to insure an excess. Filter, evaporate, add 5 ml of  $CHCl_3$ , evaporate, dry at  $110^{\circ}$  for 1 hour, cool, and weigh rapidly. Weight = aloin in aliquot taken.

As a check, acetylate the aloin. This may be done by dissolving in acetic anhydride (ca 10 ml), adding excess (ca 2 g) of powdered anhydrous Na

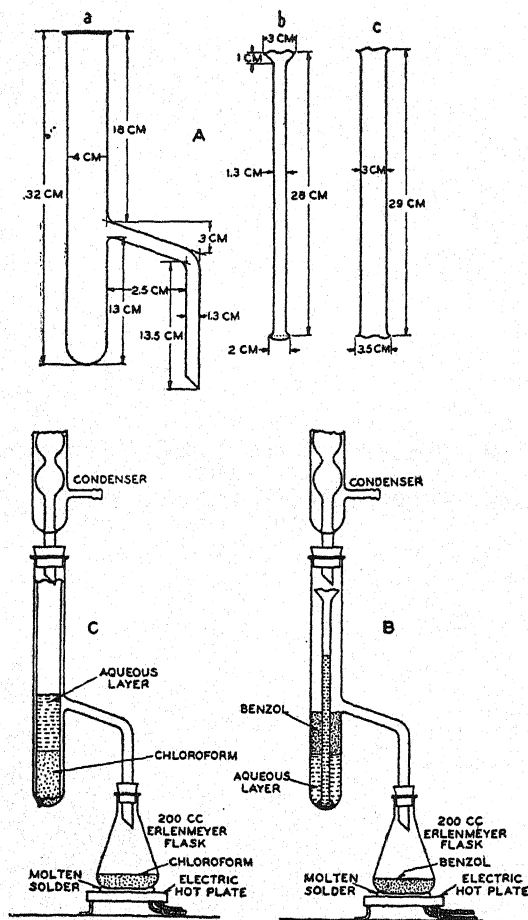


FIG. 57.—CONTINUOUS EXTRACTION APPARATUS

ing in acetic anhydride (ca 10 ml), adding excess (ca 2 g) of powdered anhydrous Na

acetate and boiling 5 min. in an acetylation flask placed in oil bath. Wash sample from flask with additional acetic anhydride and evaporate to apparent dryness in hood with good draft. Add 10 ml of  $H_2O$  and heat several minutes. Transfer with aid of  $CHCl_3$  to a separator, washing flask with successive portions of  $CHCl_3$ , and shake out with two additional 10 ml portions of  $CHCl_3$  (the aloin hexaacetate formed is soluble in  $CHCl_3$ ). Combine the  $CHCl_3$  portions, filter, evaporate, add 10 ml of  $CHCl_3$ , evaporate, dry at  $110^\circ$  for 1 hour, cool, and weigh.  $Weight \times 0.615 = \text{aloin}$ .

CASCARA SAGRADA<sup>55</sup>—TENTATIVE

109

## REAGENT

*Sodium bicarbonate soln.*—(5+100). Make up in cold  $H_2O$  as needed; add 1 ml of 0.1 N HCl to insure freedom from  $Na_2CO_3$ .

110

## DETERMINATION

Introduce  $CHCl_3$  into the continuous extraction apparatus (A, Fig. 57) to within 5 cm of the overflow. Adjust a 200 ml Erlenmeyer flask carrying 125 ml of  $CHCl_3$  to the apparatus with a well-fitted, tin-foiled cork. Into the inner tube of the apparatus introduce a measured or weighed portion of the sample representing ca 2 g of cascara sagrada. Add 20 ml of  $H_2O$  and 1 ml of acetic acid (1+100) to the cascara layer. Connect apparatus to condenser. (The outlet of condenser should not be constricted. If it is, place a hole in side near its tip to insure free return of  $CHCl_3$ .) Adjust burner, using asbestos ring to prevent overheating, and reflux rapidly 2 hours. (The  $CHCl_3$  in the tube will be colorless.) Disconnect flask and discard its contents. Recharge the Erlenmeyer flask with 125 ml of  $CHCl_3$  and connect to apparatus, which still carries the  $CHCl_3$ -exhausted acetic acid soln of the original sample and the clear exhausted  $CHCl_3$ . Add 10 ml of  $H_2SO_4$  (1+1) to the cascara layer by means of a pipet.

Connect apparatus to condenser, adjust burner, and reflux rapidly. At end of 3 hours the  $CHCl_3$  in apparatus should be practically colorless, but it may contain a small quantity of color, a non-emodin material. Remove flame and disconnect flask. Transfer the  $CHCl_3$  in flask to a separator, wash flask with 10 ml of  $H_2O$ , and transfer the  $H_2O$  to the separator carrying  $CHCl_3$ . Shake, withdraw the  $CHCl_3$ , and again wash the  $H_2O$  with 10 ml of  $CHCl_3$ , adding washings to main  $CHCl_3$  soln. Wash the  $CHCl_3$  with three 10 ml portions of the  $NaHCO_3$  soln, then wash the combined reagent with  $CHCl_3$  two or three times. Discard the aqueous soln.

Shake out the combined  $CHCl_3$  to exhaustion with saturated  $Na_2CO_3$  soln in a train of separators (four 10 ml portions should suffice). Wash the combined reagent with  $CHCl_3$  several times. Discard all the  $CHCl_3$ .

Add sufficient HCl (1+1) to the aqueous soln (cautiously, a few mls at a time) to insure an acid reaction. Extract with  $CHCl_3$  in a separator or automatic extractor to completion. Combine the  $CHCl_3$  and wash with 5 ml of  $H_2O$ . Filter the  $CHCl_3$  thru filter wetted with  $CHCl_3$ . Evaporate to 20 ml. Transfer residue to small glass or Pt dish, evaporate to dryness, and dry at  $100^\circ$  for 2 hours. Cool, and weigh the hydrolyzed products from the anthraglucosides of cascara.

111

TOTAL ALKALOIDS IN EPHEDRA<sup>56</sup>—OFFICIAL

Place 10 g of ephedra, in fine powder, in an Erlenmeyer flask. Add exactly 100 ml of solvent consisting of 3 volumes of ether and 1 volume of  $CHCl_3$  cooled to working temp. after mixing. Stopper securely, shake, and allow to stand at least 5 min. Add

5 ml of 10%  $\text{NH}_4\text{OH}$  and 0.5 g of anhydrous  $\text{Na}_2\text{CO}_3$ , stopper tightly, and macerate at least 4 hours, with occasional shaking. Decant or filter rapidly a 50 ml aliquot of the clear supernatant liquid representing 5 g of the drug, transfer to a separator, and shake with 3 portions of 2%  $\text{H}_2\text{SO}_4$ , using 15, 10, 10 ml, etc., until extraction is complete. Combine the acid solns in a separator, neutralize with  $\text{NH}_4\text{OH}$ , and add ca 5 g of anhydrous  $\text{Na}_2\text{CO}_3$ , stirring until dissolved. Shake with 5 portions of ether, using 35, 30, 25, 20, and 15 ml, until extraction is complete, and combine the ether portions in a second separator. When clear, decant and filter into a small beaker thru pledget of cotton previously wet with ether.

Evaporate the solvent to 5 ml on steam bath with aid of fan, and add bromothymol blue indicator, VI, 117(e), and a measured excess of 0.02  $N$   $\text{H}_2\text{SO}_4$ . Cover with watch-glass, return to steam bath in order to dissolve any alkaloid adhering to sides of beaker, and then evaporate the ether. Titrate excess acid with 0.02  $N$  alkali. 1 ml of 0.02  $N$  acid = 0.0033 g of ephedra alkaloids.

#### IDENTIFICATION OF GUMS<sup>7</sup>—TENTATIVE

112

##### REAGENTS

(a) *Chlorzinciodide*.—To 100 ml of a soln of  $\text{ZnCl}_2$ , sp. gr. 1.8, add a soln of 10 g of KI and 0.15 g of I in 10 ml of  $\text{H}_2\text{O}$ . Keep a few crystals of I in the soln.

(b) *Ruthenium red*.—To a few ml of a 10% soln of Pb acetate add enough ruthenium red to produce a wine red color.

(c) *Methylene blue*.—0.1% soln in alcohol.

(d) *Methylene blue*.—0.1% soln in  $\text{H}_2\text{O}$ .

113

##### PREPARATION OF SAMPLES

*Controls*.—Moisten 1 g of the dry gum with alcohol, add 100 ml of  $\text{H}_2\text{O}$  with constant stirring, and bring to boil. To 5 or 10 ml of the resulting liquid or jelly, add 4 volumes of alcohol, mix, and centrifuge to bring the precipitate together as a compact mass. (Some gums, notably acacia and agar, may fail to be thrown down by this treatment. The addition of a few drops of a saturated salt soln should cause rapid flocculation and settling.)

*Jellies or lotions*.—Stir, and add  $\text{H}_2\text{O}$  if necessary to produce a fluid mass. Treat a portion of the sample with alcohol to precipitate the gum as directed under *Controls*. Remove fatty or oily material, if present, by washing the precipitated gum with ether, then redissolve in  $\text{H}_2\text{O}$  and re-precipitate.

114

##### DETERMINATION

With a clean towel squeeze a small lump of the alcohol precipitate against a slide to form a mat 4–8 mm in diameter on the slide. Note character of resulting mat as a possible index to the type of gum. Quince and Irish moss form thin and rather translucent films while agar, starch, and acacia are white and opaque. Cover mat with a large drop of the chlorzinciodide soln and observe carefully both with and without magnification. For direct examination place slide upon white surface. For microscopic examination use a magnification of ca 90 diameters. If no characteristic color is produced within 1–2 min., proceed with a fresh mat to examine for the group (table). Continue in a similar manner thru all the group tests or until the identity is established. Use a fresh mat for each individual test.

*Characteristics of test for gums*  
Group I.—Reagent Chlorzinciodide

GUM	ORIGINAL ALCOHOL PPT.	GROUP REACTION	CONFIRMATORY TEST	REMARKS
Tragacanth	Stringy Bluish Translucent	Blue	Warm with 10% NaOH on steam bath Yellow	Certain gums, e.g., Irish moss, may yield dull yellow color with NaOH. Tragacanth bright yellow
Starch	White Compact	Blue black	Iodine, 0.1 N Blue	Tragacanth may yield faint blue
Quince	Stringy Translucent	Blue	Above tests negative	Quince is distinguished from starch and tragacanth by negative reactions
Irish moss	Stringy	Brown (small blue particles)	Characteristic nodular structures with group reagent	Old preparations of this gum may fail to show characteristic structures

Group II.—Reagent Tincture of Iodine U.S.P.

(Allow tincture to dry on mat, flush off with alcohol, and irrigate with H<sub>2</sub>O.)

Agar	White opaque	Opaque blue black	Stains with ruthenium red	Does not dissolve or lose shape when covered with H <sub>2</sub> O
Irish moss	Stringy	Brown or lilac	Characteristic blue stain with alcoholic methylene blue	These reactions yielded by old as well as fresh preparations

Group III.—Reagent Ruthenium Red

Karaya	Fine flocculent compact mass on centrifuging	Swells considerably. Strongly stained pink granular mass	Heat with conc. HCl. Pink	Aqueous methylene blue produces a characteristic blue stain
--------	----------------------------------------------	----------------------------------------------------------	---------------------------	-------------------------------------------------------------

Group IV.—Reagent Concentrated H<sub>2</sub>SO<sub>4</sub>  
(Warm cautiously on steam bath.)

Galagum	Stringy	Pink or red brown	No satisfactory test found	Alcohol precipitate from galagum resembles that from tragacanth
Acacia		Greenish brown	Ppt. completely soluble in H <sub>2</sub> O	Complete soln of acacia distinguishes it from most other gums



## TOTAL ALKALOIDS IN IPECAC, FLUIDEXTRACT

*Automatic Extraction Method<sup>58</sup>—Tentative*

116

## PREPARATION OF SOLUTION

Pipet 20 ml of the fluidextract into a 100 ml volumetric flask, add ca 5 ml of normal  $\text{H}_2\text{SO}_4$ , and with the aid of an air blast evaporate on steam bath to a volume of ca 10 ml. Then, while rotating flask, add ca 30 ml of  $\text{H}_2\text{O}$ , cool to room temp., and make up to mark with  $\text{H}_2\text{O}$ . Allow to stand overnight and filter thru dry filter, rejecting first few ml of filtrate.

117

## DETERMINATION

Measure 20 ml of prepared filtrate (4 ml of fluidextract of ipecac) into an automatic extractor (B, Fig. 57), which has been fitted to a 200 ml Erlenmeyer flask. Add 60 ml of  $\text{H}_2\text{O}$ , 2 ml of 8%  $\text{NH}_4\text{OH}$  soln, and ca 50 ml of peroxide-free ether. Shake gently to prevent deposition of any solid matter on bottom of extractor and then add peroxide-free ether until ca 75 ml has passed over into flask. Heat flask on steam bath (not electric hot plate) and extract 2 hours, or until extraction is complete. Separate ether from aqueous layer and add it to main concentrate in flask. Evaporate combined ether extract on steam bath, add 2–3 ml of absolute alcohol, and repeat evaporation to remove all traces of  $\text{NH}_3$ . Warm the alkaloidal residue on steam bath with 2–3 ml of neutral alcohol to insure complete soln. Add 10 ml of 0.1 N  $\text{H}_2\text{SO}_4$ , and dilute with ca 20 ml of recently boiled, cooled  $\text{H}_2\text{O}$ . Titrate the excess of acid with 0.02 N  $\text{NaOH}$ , using methyl red as indicator. 1 ml of 0.1 N  $\text{H}_2\text{SO}_4$  = 0.024 g of ether-soluble alkaloids of ipecac.

118

*Hand Extraction Method—Tentative*

(More rapid than automatic extraction method and yields results almost as high.)

Pipet 20 ml of prepared filtrate, 116, into a separator. Add 2 ml of 8%  $\text{NH}_4\text{OH}$  soln and extract the soln with equal volumes of peroxide-free ether until extraction is completed (at least 8 times), using Mayer's reagent, XXXIII, 29(k), as a test. Wash the combined ether extracts in a second separator with ca 10 ml of  $\text{H}_2\text{O}$ , and then wash this wash  $\text{H}_2\text{O}$  with a little peroxide-free ether, adding ether washings to main soln. Transfer ether soln to Erlenmeyer flask (200 ml flask is convenient size), and evaporate the ether on steam bath with aid of blast of air. Add 2–3 ml of absolute alcohol and repeat evaporation to remove all traces of  $\text{NH}_3$ . Warm the alkaloidal residue with 2–3 ml of neutral alcohol to insure complete soln, and titrate as directed in 117.

PODOPHYLLUM<sup>58</sup>—TENTATIVE

119

## DETERMINATION OF RESIN

Place 10 g of sample, No. 60 powder, in an Erlenmeyer flask of ca 250 ml capacity and add 35 ml of alcohol. Fit the flask with stopper thru which is inserted a glass tube ca 1 m long to act as a condenser, and heat mixture on gently simmering steam bath 30 min., shaking occasionally. Transfer contents of flask to a small percolator and percolate slowly with hot alcohol until ca 95 ml of percolate has been obtained. Collect ca 10 ml more of the percolate in a separate container. Cool first percolate to room temp. and make up volume to 100 ml with a portion of second percolate.

Place 50 ml of the alcoholic soln in a tared beaker and add 2 ml of  $\text{H}_2\text{O}$ . Evaporate until percolate weighs 3 g. If weight should fall below 3 g, add alcohol dropwise to make up to 3 g. Pour residue slowly, with constant stirring, into a second beaker containing 10 ml of  $\text{H}_2\text{O}$  previously mixed with 1 ml of normal  $\text{HCl}$  and cooled to a temp. below  $10^\circ$ . (Pellets of ice placed in the beaker and renewed from time to time serve well.) Add 5 ml of  $\text{H}_2\text{O}$  and a few drops of 10%  $\text{HCl}$  to the tared beaker, stir

well, and rub sides of container with glass rod. Add mixture to the second beaker and allow to stand overnight in refrigerator. Decant supernatant liquid into a tared Gooch crucible and transfer precipitate to crucible by means of small portions of cold  $\text{H}_2\text{O}$  slightly acidulated with  $\text{HCl}$ . (If preferred, collect precipitate on filter paper and, after washing, dissolve in hot alcohol, collecting soln in tared beaker.) Dry contents of crucible at  $80^\circ$  and weigh. If particles of resin adhere to either beaker, dissolve them in alcohol, evaporate solvent in tared beaker, and dry residue at  $80^\circ$ . Cool, weigh, and add total net weight to weight of contents of crucible.

120

BELLADONNA AND STRAMONIUM OINTMENTS<sup>60</sup>—TENTATIVE*Method I*

Introduce 25 g of the well-mixed ointment into a 250 ml separator fitted with pledget of cotton packed loosely in stem; add 100 ml of ether- $\text{CHCl}_3$  mixture (4+1) and shake vigorously until all fats are dissolved. Extract the alkaloids by shaking out with 5 successive 20 ml portions of dilute  $\text{H}_2\text{SO}_4$  (2% is satisfactory), allow to settle, and draw off clear acid soln into small separator containing 10 ml of ether. Wash each acid extraction successively thru this same 10 ml of ether and draw off the acid solns into another 250 ml separator. Make the combined acidified solns alkaline with ammonia water and extract alkaloids completely by shaking out with 5 successive 25 ml portions of  $\text{CHCl}_3$ , allow to settle, and filter each portion thru cotton wetted with  $\text{CHCl}_3$  into a 250 ml beaker, finally washing stem of separator and filter with a little  $\text{CHCl}_3$ . Evaporate solvent carefully on water bath with moderate heat to volume of ca 10 ml; add measured excess (ca 10 ml) of 0.02  $N$   $\text{H}_2\text{SO}_4$ , stir mixture, and continue evaporation until all  $\text{CHCl}_3$  has been expelled. Add 20 ml of recently boiled, cooled  $\text{H}_2\text{O}$  and one drop of methyl red indicator and titrate the excess acid with 0.02  $N$   $\text{NaOH}$ . 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.00578 g of the alkaloids of belladonna or stramonium leaves.

121

*Method II*

Weigh ca 25 g of the ointment into a tall-form beaker. Add ca 5 g of paraffin, 25 ml of 2%  $\text{H}_2\text{SO}_4$ , and 10 ml of ether. Warm gently on steam bath until fluid, stirring mixture thoroly. Continue this procedure until most of ether has been evaporated. Place beaker in ice bath and allow to stand until cold. Make several holes in paraffin layer with stirring rod and filter acid soln thru pledget of cotton into a small separator. Wash the cake once with small quantity of  $\text{H}_2\text{O}$ , filtering washings thru cotton into separator. Wash the acid with 10 ml of ether and draw off into a 250 ml separator. Repeat treatment with 4 successive portions of acid and ether, filtering each portion thru the cotton into the small separator and washing each extraction with the same 10 ml portion of ether. Combine the acidified extractions, make alkaline with 10%  $\text{NH}_4\text{OH}$ , and extract and titrate the alkaloids as directed in 120.

NOTE: It is recommended that the ointment be transferred by means of a soft metal ointment tube or empty tooth paste tube, and weighed by difference. The assay can be hastened by centrifuging when instructions are given to let the mixture stand until it settles.

122

MENTHOL<sup>61</sup>—OFFICIAL

Weigh 5 g of menthol in a 100 ml acetylation flask, and add 10 ml of acetic anhydride and 1 g of powdered anhydrous  $\text{Na}$  acetate. Boil mixture gently 1 hour, cool, and disconnect flask from condenser, transferring mixture to a small separator. Rinse acetylation flask with 3 successive 5 ml portions of warm  $\text{H}_2\text{O}$  and add rinsings to separator. When the liquids have completely separated, remove aqueous layer, and wash remaining oil with successive portions of  $\text{Na}_2\text{CO}_3$  soln (12.5 g in

100 ml of  $H_2O$ ), diluted with equal volume of  $H_2O$ , until mixture is alkaline to 2 drops of phenolphthalein soln. Dry resulting oil with fused  $CaCl_2$  and filter. Transfer 4–5 ml of the dry acetylated oil to a tared 100 ml Erlenmeyer flask, note exact weight, add 50 ml of 0.5 *N* alcoholic KOH, connect flask to reflux condenser, and boil mixture on a water bath 1 hour. Allow mixture to cool, disconnect flask from condenser, and titrate the excess of alkali with 0.5 *N*  $H_2SO_4$ , using 10 drops of the phenolphthalein soln as indicator. Calculate percentage of menthol by following formula:

$$\text{Percentage of total menthol} = \frac{A \times 7.808}{B - (A \times 0.021)}, \text{ in which}$$

*A* is result obtained by subtracting number of ml of 0.5 *N*  $H_2SO_4$  required in titration from number of ml of 0.5 *N* alcoholic KOH originally taken, and *B* is weight of acetylated oil taken.

#### THYMOL<sup>22</sup>—OFFICIAL

123

##### PREPARATION OF SOLUTION

Weigh 2 g of pulverized thymol, transfer to a 500 ml volumetric flask, and add 25 ml of 25% NaOH soln. Agitate until the thymol is dissolved and dilute to mark at 20° with  $H_2O$ .

##### DETERMINATION

124

##### Method I

Transfer a 25 ml aliquot of the thymol soln to a 250 ml glass-stoppered Erlenmeyer flask, add 20 ml of hot HCl (1+1), and immediately run in 1–3 ml less than the theoretical amount of 0.1 *N* Br soln, 26(c). Warm to 70–80°, add 2 drops of methyl orange soln (0.1 g in 100 ml of  $H_2O$ ), and titrate slowly with the Br soln, swirling vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating soln, stopper, shake vigorously 10 seconds, add a drop of the methyl orange soln, and again shake vigorously 10 seconds. Continue the addition of 2 drops of the Br soln, shaking until the red color disappears. Add 1 drop of the methyl orange soln, shake vigorously, and if the red color does not disappear, repeat the alternate addition of 2 drops of Br soln and 1 drop of methyl orange soln, shaking after each addition as directed previously, until the red color disappears. Calculate number of ml of Br soln used to percentage of thymol. 1 ml of 0.1 *N* Br soln = 0.003753 g of thymol. Reserve mixture in titrating flask for *Method II*.

125

##### Method II

To the cooled mixture resulting from the titration, *Method I*, add 3–5 ml additional Br soln. Stopper, shake, add 1 g of solid KI, wash sides of flask and stopper with  $H_2O$ , and titrate the I liberated by the excess Br soln with 0.1 *N* thiosulfate soln, using starch soln, VI, 3(e), as indicator. Calculate amount of thiosulfate used in terms of Br soln, deduct from total amount of Br soln added, and calculate to percentage of thymol.

To determine approximate number of ml of Br soln required for *Method I*, heat a 25 ml aliquot of the sample and 20 ml of HCl (1+1) to ca 80° and titrate slowly with the Br soln, swirling vigorously while titrating until a yellow color, permanent for 1 min., appears.

126

#### THYMOL IN ANTISEPTICS<sup>23</sup>—OFFICIAL

If alcoholic content is not known, make preliminary determination of alcohol.

Transfer 50 ml of sample (or aliquot containing 0.05–0.10 g of thymol) to a Pt or porcelain evaporating dish. Add 6–7 ml of 50% NaOH soln, mix well, and carefully dealcoholize by placing dish on steam bath before electric fan. Evaporate a volume

slightly more than quantity of alcohol present. (If over 30% of alcohol is present, dilute with  $H_2O$  to an alcoholic content of 25%. In no case should the evaporation be carried beyond 70% of original volume.) Transfer soln to a 125 ml separator, washing out evaporating dish with sufficient  $H_2O$  to bring the volume to ca 75 ml.

Extract the alkaline soln twice with petroleum benzin, using 20 ml each time. Wash the extracts once with 5–10 ml of 5% NaOH soln and add washings to aqueous layer. Extract aqueous alkaline soln containing the thymol, together with Na salts of boric, benzoic, and salicylic acids, with ethyl ether, making 5 extractions (20, 15, 15, 10, 10 ml). Use 8 to 10 extractions if the preparation contains glycerol. Combine the ether extracts, transfer to a 250 ml glass-stoppered Erlenmeyer flask, add 5 ml of recently prepared alcoholic KOH soln, XXXI, 24, and evaporate most of the ether, using steam bath and electric fan. Do not evaporate entirely to dryness but leave 6–8 ml residue. To this residue add 75 ml of hot  $H_2O$  (80–90°) and 10 ml of HCl.

Immediately run in 1–3 ml less than the theoretical quantity of 0.1 *N* bromide-bromate soln, 26(c), swirling contents of flask constantly. Add 2 drops of methyl orange soln and titrate slowly with the bromide-bromate soln, shaking vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating soln, stopper, shake vigorously 10 seconds, add one drop of methyl orange soln, and again shake vigorously 10 seconds. Continue the addition of bromide-bromate soln, 2 drops at a time, and shake after each addition until red color disappears. Add 1 drop of methyl orange soln, shake vigorously, and if the red color does not disappear, repeat alternate addition of 2 drops of the bromide-bromate soln and 1 drop of methyl orange soln, shaking after each addition, as directed above, until red color disappears. 1 ml of 0.1 *N* bromide-bromate = 0.003753 g of thymol.

Test for complete extraction by shaking out the aqueous layer twice with 15–20 ml of ether and titrating the thymol, if any, in the ether extracts. Add this titration to that obtained for the main ether extract. If the theoretical amount of thymol present is not known, add 2 drops of methyl orange soln, and titrate slowly, swirling constantly during the addition of bromine soln until the red color is bleached. Continue according to method outlined, beginning “add 2 drops of the titrating soln, stopper, and shake vigorously.”

CAUTION: To avoid loss of thymol by volatilization, both the evaporation of alcohol and later evaporation of ether must be done carefully.

## 127

VOLATILE ACIDITY OF TRAGACANTH<sup>64</sup>—TENTATIVE

The quantity of volatile (acetic) acidity developed in the acid hydrolysis of gum tragacanth (*Astragalus gummifer* Lab.) affords a valuable index of purity of this commodity when compared with results obtained by similar treatment of so-called “Indian gum” (*Cochlospermum gossypium* D. C. and *Sterculia urens* Roxb.).

Treat 1 g of whole or powdered sample in 700 ml round-bottomed, long-necked flask in the cold with 100 ml of  $H_2O$  and 5 ml of  $H_3PO_4$  for several hours, or until the gum is completely swollen. Boil gently for 2 hours under reflux condenser. A very small quantity of cellulose substance will remain undissolved. Tragacanth yields a practically colorless soln. Indian gum gives a pink or rose soln. This reaction may be used as a preliminary test for detection of Indian gum.

Distil the hydrolyzed product with steam, using a scrubber (Fig. 53) to connect distillation flask with condenser. Continue distillation until distillate amounts to 600 ml, and the acid residue to ca 20 ml. To avoid scorching of residue do not permit concentration of contents of distilling flask to less than 20 ml. Titrate distillate with 0.1 *N* NaOH soln, using 10 drops of phenolphthalein indicator, II, 10(d). Correct

result by blank determination and express as "volatile acidity" the number of ml of 0.1 N NaOH soln required to neutralize the volatile (acetic) acid obtained.

128

CHLOROBUTANOL<sup>65</sup>—TENTATIVE

## REAGENTS

- (a) *Alcoholic potassium hydroxide soln.*—See 130(a).  
(b) *Silver nitrate soln.*—Dissolve 10 g of AgNO<sub>3</sub> in sufficient H<sub>2</sub>O to make 500 ml.

129

## DETERMINATION

(a) *Chlorobutanol crystals.*—Transfer to a pressure bottle a sample equivalent to ca 0.3 g of chlorobutanol and carefully add 25 ml of the alcoholic KOH soln. Stopper bottle, and mix contents by gentle swirling, taking care to prevent the soln from coming in contact with rubber washer, then allow to stand 30 min. or overnight. Place bottle in wire basket, and set basket in water bath at room temp. Invert a tin can over bottle and cover with a towel to prevent injury in case bottle should burst. Heat bath to boiling and maintain this temp. 15 min. Cool gradually.

Add 25 ml of H<sub>2</sub>O, swirling gently; and transfer contents of pressure bottle to 400 ml beaker. Wash bottle with H<sub>2</sub>O, draining washings into beaker. Add 15 ml of HNO<sub>3</sub> and an excess of the AgNO<sub>3</sub> soln, stir well, and allow mixture to stand in dark place 15 min. Collect precipitate in Gooch crucible that has been dried at 105° and weighed. Wash precipitate thoroly with H<sub>2</sub>O, then with 5 ml of alcohol followed by a 5 ml portion of ether. Dry to constant weight at 105°. If reagents contain Cl, apply correction determined by blank test. 1 g of AgCl = 0.4127 g of C<sub>4</sub>H<sub>7</sub>OCl<sub>3</sub>.

(b) *Ampul solns.*—Pipet into a distilling flask a sample equivalent to ca 0.1 g of chlorobutanol. Add sufficient H<sub>2</sub>O to bring volume to 50 ml and distil ca 25 ml thru a straight-bore condenser. Collect distillate in pressure bottle of ca 100 ml capacity containing 25 ml of the alcoholic KOH and surrounded by ice bath. Have delivery tube extend into the alcoholic soln. (Use a straight-bore condenser to assure complete soln of the crystals of chlorobutanol in the condenser.) Allow to cool, disconnect still head, and wash condenser carefully with 25 ml of alcohol, allowing alcohol to drain into pressure bottle. Repeat washing, using ca 20 ml of H<sub>2</sub>O. Also wash the receiving tube with H<sub>2</sub>O. Stopper pressure bottle and mix contents by gentle swirling, taking care to prevent soln from coming in contact with rubber washer. Allow to stand 30 min. or overnight. Complete determination of Cl as directed in (a).

130

CHLOROFORM IN MIXTURES<sup>66</sup>—TENTATIVE

(Non-volatile chlorides present.)

## REAGENTS

- (a) *Alcoholic potassium hydroxide.*—Dissolve 35 g of KOH (free from chloride) in sufficient methyl alcohol to make 100 ml (saturated soln). Decant after several days.  
(b) *Silver nitrate soln.*—See 128(b).  
(c) *Phenolphthalein soln.*—Dissolve 1 g in sufficient alcohol to make 100 ml.

131

## DETERMINATION

Place 0.1 g of CaCO<sub>3</sub> and 75 ml of alcohol in a 250 ml Kjeldahl distilling flask. Pipet into this mixture 20 ml of sample, being careful to keep tip of pipet just below surface of liquid and not to agitate mixture. Add a few fragments of carborundum and connect flask with a straight-bore condenser. Distil into a previously cooled pressure bottle immersed in cracked ice and containing 25 ml of the alcoholic KOH soln into which the tip of the delivery tube should extend.

When 70 ml of the alcohol has distilled over (bottle marked previously), discontinue distillation and wash receiving tube with ca 10 or 15 ml of  $H_2O$ , collecting washings in the pressure bottle. Stopper bottle; gently agitate, taking care to prevent soln from coming in contact with rubber washer; and allow to stand overnight at room temp. Heat on steam bath 1 hour, remove from bath, and allow to cool. Empty contents of bottle into a 500 ml beaker and wash bottle with  $H_2O$  until washings are no longer alkaline to phenolphthalein, adding each washing to main soln. Add 15 ml of  $HNO_3$  and an excess of  $AgNO_3$ , stir well, and allow mixture to stand in dark place 15 min. Collect precipitate in a tared Gooch crucible that has been dried at  $105^\circ$ . Wash the precipitate with several portions of  $H_2O$ , then with 5 ml of alcohol followed by a 5 ml portion of ether. Dry at  $105^\circ$  and weigh.

1 g of  $AgCl = 0.2776$  g of  $CHCl_3$ . If it is assumed that 1 minim of  $H_2O$  weighs 0.06161 g and that the sp. gr. of  $CHCl_3$  is 1.476 (average U.S.P. limits), then 1 minim of  $CHCl_3$  weighs 0.09094 g. If the calculations are based on 1 fl. oz. measuring 29.57 ml, the factor for g of  $AgCl$  to minims of  $CHCl_3$  per fluid ounce is 4.514 for the 20 ml sample.

#### CHLOROFORM AND CARBON TETRACHLORIDE<sup>67</sup>—TENTATIVE

132

##### REAGENTS

- (a) *Alcoholic potassium hydroxide*.—See 130(a).
- (b) *Ammonium thiocyanate soln*.—0.05 N. Adjust by titrating against 0.1 N  $AgNO_3$  soln.
- (c) *Ferric ammonium sulfate indicator*.—Dissolve 8 g of  $FeNH_4(SO_4)_2 \cdot 12H_2O$  in sufficient  $H_2O$  to make 100 ml.

133

##### WEIGHING OF SAMPLE

(1) *Chloroform or Carbon Tetrachloride*.—Carefully transfer 30 ml of alcoholic KOH soln to an air-dried, 60–70 ml pressure bottle, and stopper. Do not moisten neck of bottle with the reagent. Weigh stoppered bottle with contents (conveniently done by suspending bottle on balance by means of the clamp that holds stopper).

Immediately after opening bottle, add ca 1 ml of the sample from a 1 ml pipet, holding pipet just above top level of reagent in pressure bottle. As level of reagent rises with draining of sample into bottle, raise pipet correspondingly so as to avoid contact with reagent. Avoid having bottle open longer than necessary, 20 seconds being convenient time. Stopper bottle so as to assure a tight fit and weigh. Determine weight by difference. Proceed as directed under 134.

(2) *Carbon Tetrachloride in Capsules*.—Ascertain gross weight of representative number of capsules. Open capsules and transfer contents to a suitable flask. Weigh dried empty capsules and determine average net contents. Proceed as directed under (1), using the composite sample.

(3) *Chloroform or Carbon Tetrachloride in Mixtures*.—Proceed as directed under (1), using not more than 10 ml of the mixture containing 0.08–1.6 g of  $CHCl_3$  or  $CCl_4$ . Note temp. of mixtures. Ascertain volume-equivalent of weighed sample. Weigh definite volume of mixture at same temp., using a 50 or 100 ml volumetric flask, and calculate.

NOTE: If desired, the sample may be measured directly with a pipet instead of being weighed, or a measured volume may be diluted with methyl alcohol to some definite volume and thoroly mixed and a suitable aliquot of this dilution used.

134

##### DETERMINATION

If sample is a mixture, mix contents of bottle by gentle swirling and allow bottle to stand ca an hour (30 min. is sufficient for  $CHCl_3$ , pure or nearly so). Place bottle

in a wire basket and set basket in water bath at room temp. Invert a tin can over bottle and cover with a towel to prevent injury to analyst in case bottle should burst. Heat bath to boiling and maintain at this temp. 1 hour (15 min. is sufficient for  $\text{CHCl}_3$ , pure or nearly so). Cool contents of pressure bottle gradually, transfer to a 200 ml volumetric flask, and wash out bottle thoroly with  $\text{H}_2\text{O}$ , draining washings into the flask. Bring to room temp., fill to mark with  $\text{H}_2\text{O}$ , and mix.

Transfer a suitable aliquot to a 100 ml volumetric flask and acidify with  $\text{HNO}_3$ , adding ca 2 ml in excess. Add 25 or 50 ml of 0.1  $N$   $\text{AgNO}_3$  (an excess), shake thoroly, fill to mark with  $\text{H}_2\text{O}$ , and mix. Filter mixture thru dry filter into dry flask, rejecting first 20 ml of filtrate. To a 50 ml aliquot of filtrate, add 3 ml of the  $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2$  indicator and titrate the excess 0.1  $N$   $\text{AgNO}_3$ , using 0.05  $N$   $\text{NH}_4$  or  $\text{K}$  thiocyanate.

If the original sample contains chloride, determine the quantity and make correction. If the original sample contains sugar or other organic material and (after saponification of the  $\text{CHCl}_3$  or  $\text{CCl}_4$  and dilution of mixture with  $\text{H}_2\text{O}$ ) is highly colored, thus interfering with titration, transfer contents of pressure bottle to a Ni crucible with the aid of  $\text{H}_2\text{O}$ . Evaporate to dryness and char residue. Allow to cool, treat with  $\text{H}_2\text{O}$ , filter into a suitable volumetric flask and wash residue and filter with  $\text{H}_2\text{O}$  until free from chloride. Fill to mark with  $\text{H}_2\text{O}$ , mix, and determine the chloride as directed previously.

Make a blank test, using in pressure bottle the same quantities of solvents and reagents as with sample, and apply necessary correction. 1 ml of 0.1  $N$   $\text{AgNO}_3$  = 0.003979 g of  $\text{CHCl}_3$  or 0.003846 g of  $\text{CCl}_4$ .

#### TETRACHLORETHYLENE IN MIXTURES<sup>68</sup>—TENTATIVE

135

##### REAGENTS

(a) *Metallic sodium*.—Place 10 ml of xylene and 2 g of metallic  $\text{Na}$  in a small Erlenmeyer flask fitted with glass stopper, adding more xylene if necessary to cover metal. Heat on hot plate until the  $\text{Na}$  is melted. Shake to remove excess vapor, stopper, wrap in towel, and shake vigorously until the  $\text{Na}$  is finely divided. Cool, remove xylene, and replace with 5 ml of fresh xylene.

(b) *Ferric ammonium sulfate indicator*.—See 132 (c).

136

##### DETERMINATION

Weigh carefully a 125 ml cork-stoppered Erlenmeyer flask. Remove from balance pan, open, and from a split ml pipet add sufficient sample to give equivalent of ca 0.16 g of tetrachlorethylene. Stopper securely and weigh again. To contents of flask add 10 ml of xylene and 2 g of the  $\text{Na}$  reagent. Connect flask to reflux condenser, using cork stopper protected by tin foil, and heat on hot plate to boiling. Add ca 1 ml of amyl alcohol thru condenser. Reflux gently 2 hours and add at intervals 1 ml portions of amyl alcohol until a total of 5 ml is added. Disconnect flask. When cool, destroy excess of  $\text{Na}$  by cautious addition of 20 ml of  $\text{H}_2\text{O}$ . After all action has subsided, acidify with  $\text{HNO}_3$  and transfer mixture to a separator. Wash xylene layer with three 10 ml portions of  $\text{H}_2\text{O}$  and filter the acid aqueous solns into a 200 ml volumetric flask. Add 50 ml of 0.1  $N$   $\text{AgNO}_3$  soln to flask and make up to 200 ml. Shake thoroly and pour thru a dry filter, discarding first 20 ml of filtrate. To a 100 ml aliquot add 3 ml of the indicator. Titrate excess  $\text{AgNO}_3$ , using 0.05  $N$   $\text{NH}_4\text{CNS}$ . Make a blank test for chloride. 1 ml of 0.1  $N$   $\text{AgNO}_3$  = 0.004146 g of  $\text{C}_2\text{Cl}_4$ . The chloride may also be determined gravimetrically. 1 g of  $\text{AgCl}$  = 0.2892 g of  $\text{C}_2\text{Cl}_4$ .

#### ETHER<sup>69</sup>—OFFICIAL

(Not applicable in presence of essential oils.)

137

## REAGENTS

(a) *Sulfuric acid*.—(1+1). Carefully add  $\text{H}_2\text{SO}_4$  to an equal volume of  $\text{H}_2\text{O}$  and cool to room temp.

(b) *Potassium dichromate soln*.—1 N. Dissolve 49.035 g of pure  $\text{K}_2\text{Cr}_2\text{O}_7$  (or corresponding quantity of known purity) in sufficient  $\text{H}_2\text{O}$  to make 1 liter.

(c) *Sulfuric acid-potassium dichromate soln*.—0.5 N. Carefully add 500 ml of  $\text{H}_2\text{SO}_4$  to 500 ml of 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  soln (accurately measured in a volumetric flask), and cool to room temp. Use two 1 liter flasks for mixing and cooling. Transfer to a 1 liter volumetric flask, using  $\text{H}_2\text{SO}_4$  for washing, and fill to mark with  $\text{H}_2\text{SO}_4$ . Mix thoroly.

Standardize against 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$  soln as follows:

Pipet exactly 25 ml of Reagent (c) into a 250 ml ground-glass-stoppered volumetric flask and dilute to mark with  $\text{H}_2\text{O}$  after cooling to room temp. Mix thoroly. Pipet a 50 ml aliquot into a 500 ml ground-glass-stoppered flask; add 100 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{H}_2\text{SO}_4$ , and 10 ml of 25% KI soln, freshly prepared. Stopper flask and allow to stand 3–5 min. Add 150–200 ml of  $\text{H}_2\text{O}$  and titrate with 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch soln, VI, 3(e), freshly prepared as indicator.

138

## APPARATUS

Set up apparatus as illustrated, Fig. 58. Beginning at air intake end of aspiration train, use a 400 ml bottle as wash bottle (A), six 50 ml graduated cylinders, having an inside diameter of 1.5 cm and a height of 32–35 cm (B–C–D–E–F–G), a 500 ml bottle as safety reservoir (H), and a 400 ml bottle as wash bottle (I), which is supplied with a soda-lime tube. Supply each container with a closely fitting rubber stopper and vapor-carrying tubes. Have the intake tube extend almost to the bottom, and the outlet tube 1 cm below the rubber stopper. Use heavy-walled glass tubing having an outside diameter of 5 mm. Draw outlets of all vapor-carrying tubes down to small openings. Use heavy walled rubber tubing for connections and between cylinders expose only 0.5–1 cm to the vapors.

139

## PREPARATION OF SAMPLE

Carefully weigh a 100 ml glass-stoppered volumetric flask containing 65–70 ml of  $\text{H}_2\text{O}$ . Pipet 5 ml of ether, holding pipet just above the  $\text{H}_2\text{O}$  in the flask, and as the level of the  $\text{H}_2\text{O}$  is raised by the draining of the ether into the flask raise pipet correspondingly to avoid contact with the  $\text{H}_2\text{O}$ . Immediately stopper flask and weigh. The difference in weight is weight of ether. Carefully and gently swirl liquid in flask until ether is dissolved and then fill to mark with  $\text{H}_2\text{O}$ . Stopper flask and thoroly mix. If the unknown ether sample is an alcoholic or hydroalcoholic soln, prepare a soln by dilution with  $\text{H}_2\text{O}$  to meet requirements under 141.

140

## PRELIMINARY CHARGING OF APPARATUS

Transfer ca 100 ml of the  $\text{H}_2\text{SO}_4$ - $\text{K}_2\text{Cr}_2\text{O}_7$  soln to wash bottle A, and 35 ml of the  $\text{H}_2\text{SO}_4$  soln to each cylinder, C and D. (Use a funnel with a long stem to avoid wetting upper portion of container.) Pipet 40 ml, 25 ml, 25 ml of the  $\text{H}_2\text{SO}_4$ - $\text{K}_2\text{Cr}_2\text{O}_7$  soln into cylinders E, F, and G, respectively, avoiding unnecessary wetting of outside of stem of pipet and touching inside of cylinder with the wetted stem of the pipet while draining. Bottle H remains empty. Transfer ca 50 ml of  $\text{H}_2\text{SO}_4$  to bottle I and fill tube J with an appropriate quantity of soda-lime, layered on bottom and top with cotton. Stopper tightly all containers except cylinder B. Leave all rubber tubing connections between cylinders and glass stopcocks K and L open.



If sample is known not to contain alcohol or other substances that will be oxidized by the  $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$  soln, pipet an aliquot as directed in 140 into a 250 ml ground-glass-stoppered flask containing 50 ml of the same reagent. Stopper flask, swirl gently, and allow to stand 1 hour. Titrate excess acid-dichromate and calculate as directed below.

Pipet an aliquot of the sample containing 0.035–0.2 g of ether in aqueous soln or hydro-alcoholic soln, containing not more than 5 g of alcohol, into cylinder B containing sufficient  $\text{H}_2\text{O}$  to make a total volume of 25 ml. Hold pipet just above top level of liquid in cylinder, and as the liquid is raised by the draining of the sample, raise pipet correspondingly so as to avoid contact with the liquid.

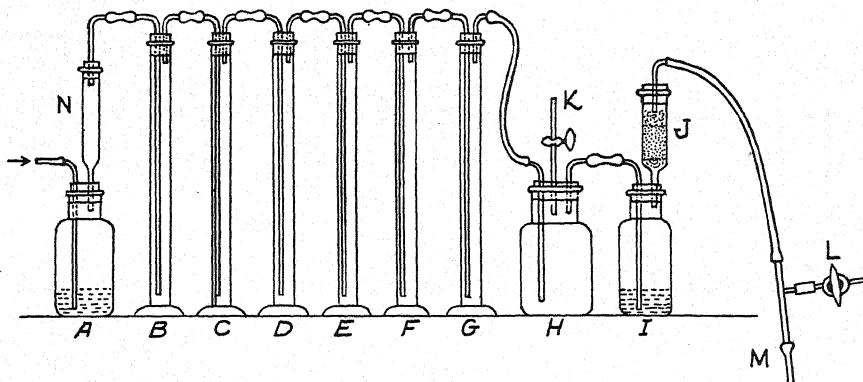


FIG. 58.—APPARATUS FOR DETERMINATION OF ETHER

Stopper tightly and immediately connect with cylinder C and wash bottle A. Connect the suction pump at M, and with stopcock L about half open start the pump. With bottle H and cylinder G connected, gradually close stopcock K until a slow current of bubbles passes thru the reagent in cylinder F, and connect cylinder E. Repeat until cylinder B, which contains the sample, is connected. Make certain all connections are air-tight. (Usually stopcock L requires no further adjustment.) Carefully adjust stopcock K until a rapid and steady current of bubbles (ca 150 per min.) flows thru the aspiration train. (Usually this is attained with cock K slightly open, depending upon size of opening thru cock L.) Use care not to have any of the reagent touch the rubber stopper by spray or otherwise. As the bubbles rise in cylinders B and C they increase in size, couple up, and near the surface each bubble occupies the entire cross-section of the cylinder and has a vertical height of 1–1.5 cm.

Aspirate for 5 hours. If not certain that all the ether has been carried over into the 0.5 *N* acid-dichromate soln, discontinue the aspiration as directed in the following paragraph. Transfer contents of cylinder E to a ground-glass-stoppered 500 ml volumetric flask. Pipet 25 ml of acid-dichromate soln into cylinder E. Aspirate as before.

Gradually open cock K until rate of flow of bubbles is appreciably slower and disconnect rubber tubing between cylinders B and C. Gradually open cock K as before and disconnect the tubing between C and D. Repeat until all cylinders are disconnected.

Transfer the acid-dichromate soln (contents of cylinders E, F and G) to a ground-

glass-stoppered 500 ml volumetric flask. Wash cylinders and glass tubes with  $\text{H}_2\text{O}$  and drain washings into flask. Add 200–300 ml of  $\text{H}_2\text{O}$  and cool. Add more  $\text{H}_2\text{O}$  and again cool to room temp. Make up to volume and mix thoroly. Pipet a 25 ml aliquot into a 500 ml ground-glass-stoppered flask and continue as directed under 137(c), beginning "add 100 ml of  $\text{H}_2\text{O}$ ." Calculate 0.5  $N$   $\text{H}_2\text{SO}_4$ - $\text{K}_2\text{Cr}_2\text{O}_7$  soln consumed by the sample. 1 ml of 0.5  $N$  acid-dichromate = 0.00463 g of ether.

## CINCOPHEN

*I. In Presence of Salicylates<sup>70</sup>—Tentative*

142

## REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 12.5 g of monohydrated  $\text{Na}_2\text{CO}_3$  in sufficient  $\text{H}_2\text{O}$  to make 100 ml.

(b) *Iodine soln.*—0.1  $N$ . See 190.

(c) *Sodium thiosulfate soln.*—0.02  $N$ . See 3(b).

(d) *Starch indicator.*—See VI, 3(e).

143

## DETERMINATION

If the product is a solid, weigh into a 50 ml beaker sufficient finely powdered sample to contain ca 0.15 g of cinchophen. Treat with 5, 3, and 3 ml portions of the  $\text{Na}_2\text{CO}_3$  soln, and filter thru a small (5 cm) paper into a 50 ml beaker, finally washing first beaker and paper with a little  $\text{H}_2\text{O}$ . Evaporate filtrate and washings to complete dryness on steam bath with aid of air blast. If the product is in the form of a clear soln, transfer a measured portion to a beaker, and evaporate to dryness. In either case, dissolve the hot residue in 5 ml of glacial acetic acid and transfer to a 100 ml volumetric flask, using not more than 10 ml of the acid to complete the transfer. Heat to ca  $90^\circ$  on steam bath. Add 25 ml of the I soln slowly from a pipet with constant agitation of flask, and immediately stopper flask. Allow to cool, dilute to 100 ml with  $\text{H}_2\text{O}$ , stopper, and let stand with occasional thoro agitation 30 min. Filter thru small, rapid filter, rejecting first 15 ml of filtrate and immediately titrate a 50 ml aliquot with the standard thiosulfate soln, adding the starch indicator as the end point is approached. 1 ml of 0.1  $N$  I = 0.0166 g of cinchophen,  $\text{C}_{16}\text{H}_{11}\text{O}_2\text{N}$ .

*II. In Presence of Sodium Bicarbonate<sup>71</sup>—Tentative*

144

## REAGENTS

(a) *Solvent.*—Mix 50 ml of alcohol and 50 ml of ether with 100 ml of  $\text{CHCl}_3$ .

(b) *Neutral alcohol.*—Neutralize to phenolphthalein with 0.1  $N$   $\text{NaOH}$ .

145

## DETERMINATION

Count, and weigh a representative number of tablets, ascertain average weight, and grind to fine powder. Weigh a sample sufficient to yield 0.3–0.4 g of cinchophen, transfer to a separator, and add 10 ml of 4%  $\text{NaOH}$  soln to dissolve the cinchophen. Neutralize with 10%  $\text{HCl}$  and add 2 ml in excess. Extract with five 25 ml portions of the solvent, collecting extracts in second separator. Wash with 25 ml of  $\text{H}_2\text{O}$  and filter extracts into beaker. Extract wash water with 15 ml of solvent and filter into same beaker. Test for complete extraction. Evaporate solvent to dryness on steam bath. Dissolve residue in 60 ml of neutral alcohol. Titrate the soln with 0.1  $N$   $\text{NaOH}$  to a permanent pink color, using phenolphthalein as indicator. 1 ml of 0.1  $N$   $\text{NaOH}$  = 0.02491 g of cinchophen.

DINITROPHENOL (OR ITS SODIUM COMPOUND)<sup>72</sup>—OFFICIAL

146

## REAGENTS

- (a) *Sodium hydroxide soln.*—Dissolve 2 g of NaOH in 100 ml of H<sub>2</sub>O.  
 (b) *Potassium iodide soln.*—Dissolve 20 g of KI in 100 ml of H<sub>2</sub>O.  
 (c) *Bromide-bromate soln.*—0.1 N. Dissolve 2.7835 g of KBr in H<sub>2</sub>O and dilute to 1 liter. If necessary, standardize against 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 26(c).  
 (d) *Sodium thiosulfate.*—0.1 N. See 3(b).

147

## DETERMINATION

(a) *Interfering substances absent.*—Weigh 0.18–0.20 g of 2,4 dinitrophenol (or sufficient of the preparation to contain that quantity) into a beaker of ca 100 ml capacity, and dissolve the substance in 25 ml of H<sub>2</sub>O, using sufficient 2% NaOH to insure soln. Transfer the soln to a 500 ml glass-stoppered flask, using H<sub>2</sub>O for washing (do not use heat). Dilute soln with H<sub>2</sub>O to ca 100 ml and add 25 ml of the bromide-bromate soln and 10 ml of HCl. Immediately stopper flask and swirl vigorously 1–3 min. Remove stopper quickly and add 5 ml of KI soln, taking care to avoid loss of bromine; immediately stopper flask and shake thoroly ca 1 min. Remove stopper and rinse down neck of flask with the H<sub>2</sub>O. Titrate with the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, using starch indicator, VI, 3(e), near the end point.

1 ml of 0.1 N bromide-bromate soln = 0.0092 g of 2,4 dinitrophenol.

1 ml of 0.1 N bromide-bromate soln = 0.0103 g of Na dinitrophenate.

1 ml of 0.1 N bromide-bromate soln = 0.0112 g of Na dinitrophenate monohydrate.

(b) *Interfering substances present.*—Weigh into a separator a sample equivalent to ca 0.18 g of 2,4 dinitrophenol or its sodium compound. Macerate for a short time with 10 ml of H<sub>2</sub>O and 10 ml of NaOH soln. Acidify with HCl. Extract with 10–20 ml of CHCl<sub>3</sub> and repeat until extraction is complete (usually 5 or 6 extractions are necessary), avoiding vigorous shaking, particularly during first 2 extractions. Test for complete extraction by shaking out the last CHCl<sub>3</sub> extraction with 5 ml of the NaOH soln (a yellow color in latter indicates incomplete extraction; 5 ml containing 0.025 mg is pale yellow).

Combine the CHCl<sub>3</sub> extracts in a separator and shake out with 10–15 ml of the NaOH soln. Draw off CHCl<sub>3</sub> layer into a third separator and repeat extraction until no more yellow color is extracted. Note total volume of NaOH soln used. Transfer the alkaline solns to a 500 ml glass-stoppered flask, washing separator each time with H<sub>2</sub>O. Add exact quantity of HCl necessary, previously determined, to neutralize the NaOH soln used. Proceed as directed under (a).

GUAIACOL<sup>73</sup>—TENTATIVE

148

## REAGENTS

(a) *Hydriodic acid.*—Sp. gr. 1.7. Boil the HI under reflux condenser with excess of hypophosphorous acid 30 min. When cool, transfer to dark, glass-stoppered bottle. Do not allow acid to stand with stopper removed for more than few minutes.

(b) *Glacial acetic acid-potassium acetate soln.*—10%. To 100 ml of glacial acetic acid add 10 g of KC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>.

(c) *Sodium acetate soln.*—25%. To 100 ml of H<sub>2</sub>O add 25 g of NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>·3H<sub>2</sub>O.

149

## APPARATUS

(1) *Boiling rod.*—Glass tube 60 mm long, 3.5 mm outside diameter, with a 1 mm

bore. It is sealed at one end and also closed ca 10 mm from the other end. The rod is placed in the flask with the open end down.

(2) *Methoxy apparatus*.—See Fig. 61, XLI.

150

## DETERMINATION

Introduce an aliquot of the alkaline guaiacol soln (guaiacol dissolved in 1% NaOH) containing 0.03–0.06 g of guaiacol into the boiling flask and evaporate the soln just to dryness on steam bath in current of air. For solid guaiacol compounds weigh 0.06–0.1 g and introduce directly into flask. Add 2.5 ml of phenol (highest quality crystalline), 5 ml of the hydriodic acid, and a boiling rod. (For solid compounds that can be weighed the same phenol and hydriodic acid will serve for many determinations. The contents of the flask A are allowed to cool, the sample is introduced, and the determination is continued.) Connect flask with remainder of apparatus, which consists of scrubber containing a little  $H_2O$  and receivers. The receivers contain 10 ml of the glacial acetic acid- $KC_2H_3O_2$  soln to which 10 drops of Br have been added. Place ca 6 ml of the soln in one and 4 ml in the other.

Pass a slow uniform stream of  $CO_2$  from a cylinder (ca 1 bubble per second) through capillary side arm of boiling flask and heat liquid gently with a micro burner at such rate that vapor of boiling liquid rises half way up condenser; after 30 min. discontinue heating but continue to pass  $CO_2$  thru apparatus for few minutes in order to carry over methyl iodide completely.

Wash contents of receivers into 250 ml Erlenmeyer flask containing 5 ml of the  $NaC_2H_3O_2$  soln. Adjust volume of liquid to ca 125 ml and add 8 drops of 90% formic acid. Rotate flask until the color due to the bromine is discharged, add 12 more drops of the formic acid, and allow flask to stand 1–2 min. Add 5 ml of 10%  $H_2SO_4$  and 1 g of KI and titrate the liberated I with 0.1 N  $Na_2S_2O_3$ . Correct for number of ml required to titrate the blank run in same way, using same quantity of reagents.

1 ml of 0.1 N I = 0.00207 g of guaiacol; 0.00228 g of guaiacol carbonate; and 0.00404 g of potassium guaiacol sulfonate.

HEXYLRESORCINOL<sup>14</sup>—TENTATIVE

151

## REAGENTS

(a) *Bromide-bromate soln*.—0.1 N. See 26(c).

(b) *Sodium thiosulfate soln*.—0.1 N. See 3(b).

(c) *Purified methanol*.—Add sufficient bromine vapor to commercial methanol to give a bright yellow color and heat to boiling on water bath 5 min. Cool, and carefully decolorize by adding 10% soln of  $NaHSO_3$  dropwise until methanol is just colorless.

(d) *Potassium iodide soln*.—Dissolve 20 g of KI in  $H_2O$  and dilute to 100 ml.

152

## STANDARDIZATION OF THIOSULFATE

Add 30 ml of the bromide-bromate soln to a 150 ml glass-stoppered flask. Add 10 ml of methanol. Wet stopper. Add 5 ml of HCl, stopper flask, immediately place under running tap  $H_2O$ , and swirl until flask cools to room temp. Continue to shake flask 5 min. after HCl is added. Cautiously loosen stopper and add 5 ml of the KI soln. Swirl gently to liberate the I, wash stopper, and titrate with the thiosulfate. Add starch paste when color of soln is a pale yellow.

153

## DETERMINATION

Transfer 0.07–0.09 g of sample to a 150 ml glass-stoppered flask. Add 10 ml of

methanol and swirl gently to dissolve sample. Add 30 ml of the bromide-bromate soln. Moisten stopper. Add 5 ml of HCl, stopper flask, and immediately hold under running H<sub>2</sub>O while swirling somewhat vigorously. When cooled to room temp. (ca 1 min.), remove from tap and continue to shake vigorously 5 min. after the HCl is added. Cautiously loosen stopper and add 5 ml of the KI soln. Swirl gently, wash stopper with a little H<sub>2</sub>O, add 1 ml of CHCl<sub>3</sub>, and titrate with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> while swirling flask gently. Near end point, stopper flask and shake vigorously to get the free halogen out of the CHCl<sub>3</sub>. When the color has been reduced to a pale yellow, add starch paste and continue titration. The end point is reached when the starch-iodide color does not return during 30 seconds of vigorous shaking. 1 ml of 0.1 N bromide-bromate soln = 0.00488 g of hexylresorcinol.

#### MANDELIC ACID<sup>75</sup>—TENTATIVE

154

##### *Qualitative Tests*

(Applicable to free acid.)

(a) Dissolve 0.25 g of sample in ca 10 ml of H<sub>2</sub>O and add a few drops of 10% FeCl<sub>3</sub> soln. A bright yellow color is produced. This is a general test for hydroxy acids and is not specific for mandelic acid.

(b) Dissolve 0.25 g of sample in 5 ml of H<sub>2</sub>O in a test tube; to soln add 5 ml of H<sub>2</sub>SO<sub>4</sub> and agitate test tube and contents a few seconds; then add 10 ml of H<sub>2</sub>SO<sub>4</sub> so as to form two layers. Agitate very gently but do not mix. A purple color slowly forms at the interface if the test tube is allowed to stand for a few minutes. A strong odor of benzaldehyde is noticed on shaking.

155

##### *Quantitative Methods*

(a) *Tablets*.—Count and weigh a representative number of tablets, ascertain average weight, and grind to fine powder. Weigh a quantity of powdered material equivalent to 0.4–0.5 g of mandelic acid and transfer to a separator containing 10 ml of H<sub>2</sub>O. Acidify with HCl (1+3) and add 2 ml of the acid in excess. Extract with six 20 ml portions of CHCl<sub>3</sub>-ether solvent (2+1); wash each portion in a second separator with 2 ml of H<sub>2</sub>O, and pass soln thru plug of cotton, previously saturated with solvent, into 250 ml beaker. Wash outer surface of stem of separator with a few ml of solvent and add this to main portion. Test for complete extraction with 15 ml more of solvent and evaporate in separate beaker. Wash any residue thus obtained into beaker containing main extract with a few ml of solvent. Evaporate to dryness at a temp. not exceeding 40° with aid of fan. Dissolve residue in 25 ml of CO<sub>2</sub>-free H<sub>2</sub>O and titrate with 0.1 N NaOH, using phenolphthalein as indicator. 1 ml of 0.1 N NaOH = 0.01521 g of mandelic acid (C<sub>6</sub>H<sub>5</sub>CHOHCOOH), 0.01691 g of NH<sub>4</sub> mandelate (C<sub>6</sub>H<sub>5</sub>CHOHCOONH<sub>4</sub>), 0.01741 g of Na mandelate (C<sub>6</sub>H<sub>5</sub>CHOHCOONa), 0.01711 g of Ca mandelate (C<sub>6</sub>H<sub>5</sub>CHOHCOO)<sub>2</sub>Ca, or 0.01632 g of Mg mandelate (C<sub>6</sub>H<sub>5</sub>CHOHCOO)<sub>2</sub>Mg.

After titration the mandelic acid may be re-extracted and the extract used for melting point determinations or qualitative tests.

(b) *Liquid preparations*.—Measure 1 ml of sample or an aliquot of a dilution sufficient to yield 0.4–0.5 g of mandelic acid into a separator and acidify with HCl (1+3). Proceed as directed in (a).

#### NICOTINIC ACID IN TABLETS AND AMPULS<sup>76</sup>—TENTATIVE

156

##### PREPARATION OF TABLET SAMPLE—See 1.

157

## DETERMINATION

(a) *Tablets*.—Weigh by difference ca 0.25 g of the powdered sample into a 500 ml suction flask (Fig. 59). Fit flask with an inside condenser consisting of 10" test tube sealed into flask by means of rubber stopper and extending to within  $\frac{1}{2}$ " of bottom. The test tube should have a clearance of ca  $\frac{1}{8}$ " on all sides in neck of flask. Connect flask thru a two-way stopcock to aspirator or vacuum line. Circulate  $H_2O$  thru condenser by means of glass tube extending to bottom of test tube. Immerse flask in a glycerin bath up to side arm and hold in this position by means of a clamp. Raise temp. of bath to ca 160°, stirring occasionally, and hold at this temp. 1 hour. Remove burner and raise flask out of bath. Cool, and turn off vacuum. Carefully fasten condenser with a clamp to a ring stand to avoid shaking off the sublimate. Break vacuum and cautiously remove flask. Wash sublimate from tube with stream of hot absolute alcohol into weighed beaker, evaporate alcohol on steam bath in current of air, and dry 20 min. at 100°. Cool in desiccator and weigh.

(b) *Ampuls*.—With a pipet measure 5 ml of sample into a 500 ml suction flask and evaporate the  $H_2O$  on steam bath with aid of current of air directed into flask. Dry residue in oven at 100° 30 min. and proceed as directed under (a), beginning "Fit flask with an inside condenser."

METHYL ALCOHOL (0.5–5.0%) IN PRESENCE  
OF ETHYL ALCOHOL<sup>77</sup>—TENTATIVE

158

## REAGENTS

*Soln. A*.—Methyl alcohol, 25% by volume ( $\pm 0.1\%$ ).

*Soln. B*.—Mix 20 ml of *Soln. A* and 95 ml of absolute ethyl alcohol (or equivalent in dilute alcohol) with  $H_2O$  to volume of 2 liters. Make all transfers and dilutions at 20°.

*Fuchsin-sulfurous acid*.—Dissolve 0.2 g of fuchsin in 120 ml of hot  $H_2O$ , cool soln, and add 2 g of  $Na_2SO_3$  in 20 ml of  $H_2O$ . Mix, add 2 ml of  $HCl$ , and dilute to 200 ml.

159

## DETERMINATION

(a) *Total alcohols*.—Measure at room temp. (20°) 25 ml of sample, add 90 ml of  $H_2O$ , neutralize to litmus with 5%  $NaOH$ , distil, and dilute volume of distillate to 100 ml at same temp. as noted when original aliquot was measured. Determine total alcohol (as ethyl alcohol) from the sp. gr. of distillate in usual way and estimate

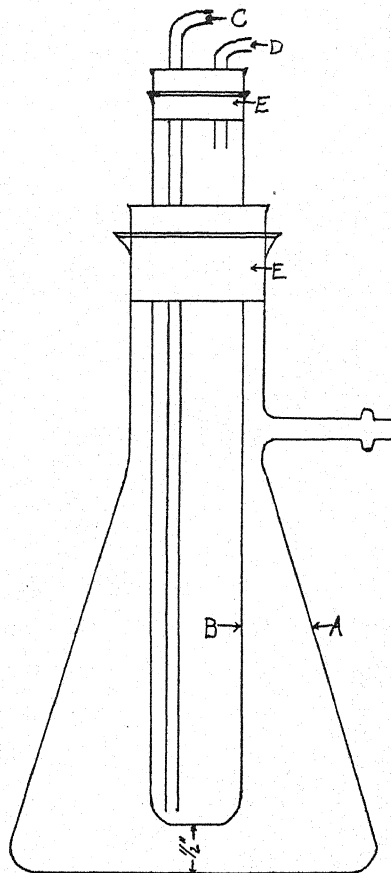


FIG. 59.—FITTED FLASK FOR DETERMINATION OF NICOTINIC ACID

percentage of alcohol in original soln by means of proper dilution factor. Test a portion of this distillate by the U.S.P. test for methyl alcohol, taking precaution to determine that  $\text{HCHO}$ , as such, is not present. If methyl alcohol is present, transfer 10 ml of distillate to a separator, add 40 ml of saturated salt soln, shake with 25 ml of petroleum benzin, and draw off the aqueous salt soln into distilling flask. Wash the petroleum benzin in the separator with two 10 ml portions of saturated salt soln, adding these to the portion already in distilling flask. Distil, receiving distillate in a 50 ml graduated flask. Calculate quantity of ethyl alcohol to add to this distillate to make a 5% soln of total alcohol (assuming it to be all ethyl alcohol) when made up to 50 ml, add this calculated amount, and make up to a volume of 50 ml. Transfer 5 ml of this distillate to a 200 ml volumetric flask for color comparison with standards.

(b) *Color standards*.—Transfer to 200 ml volumetric flasks a series of aliquots, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml of Soln B, adding 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, and 0 ml, respectively, of 5% ethyl alcohol. (These amounts of methyl alcohol represent percentages in original unknown soln when unknown is diluted as outlined above.)

(c) *Methyl alcohol*.—To each of the standards and to the unknown, add 1 ml of  $\text{H}_3\text{PO}_4$  (1+1) and 2 ml of 3%  $\text{KMnO}_4$  soln, and allow mixtures to stand 10 min. Add 1 ml of 10% oxalic acid soln and allow mixtures to stand until clear or transparent. Add 5 ml of  $\text{H}_2\text{SO}_4$  soln (1+3) and 5 ml of the freshly prepared fuchsin-sulfurous acid mixture and allow solns to stand  $1\frac{1}{2}$  hours. Dilute to 200 ml, mix thoroly, and transfer equal quantities to a series of test tubes of uniform color and diameter for color comparison. Compare the unknown with the standard which it most nearly approaches in color intensity, approximating intervals less than 0.5%, if desired. The value obtained represents the percentage of methyl alcohol in original sample.

(d) *Ethyl alcohol*.—Deduct percentage of methyl alcohol, determined colorimetrically, from percentage of total alcohols previously determined.

#### PHENOLPHTHALEIN IN TABLETS

##### *Ether Extraction Method*<sup>78</sup>—Official

(Not applicable in presence of other ether extractives.)

160

PREPARATION OF SAMPLE—See 1.

161

#### DETERMINATION

Weigh a portion of powdered material representing ca 0.2 g of phenolphthalein, transfer to a separator by means of 10 ml of 5%  $\text{NaOH}$  soln and a little  $\text{H}_2\text{O}$ , and extract 3 or 4 times with ether, using 25 ml for the first and 20 ml for each subsequent extraction. Transfer ether extracts to a second separator and wash twice with 5 ml of the 5%  $\text{NaOH}$  soln. (Substances like quinine, acetanilid, acetophenetidin as well as any unsaponified fatty material or mineral oil, if present, will be removed by extraction with ether.) Combine alkaline solns and acidify with  $\text{HCl}$ . Extract with ether as before, until all the phenolphthalein has been removed, as determined by testing a portion of the ether soln with  $\text{NaOH}$  (after 4 or 5 extractions). Filter ether extractions into a weighed beaker, evaporate, dry residue at  $100^\circ$ , and weigh. The residue should be soluble in alcohol, showing absence of most oils. If titrated with 0.1  $N$   $\text{NaOH}$ , the alcoholic soln should be practically neutral, showing absence of acid extractives, like fatty acids and salicylic or benzoic acid.

PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS<sup>20</sup>—TENTATIVE

162

## REAGENTS

(a) *Iodine soln.*—Dissolve 20 g of KI in a minimum quantity of H<sub>2</sub>O, add 14 g of I, and when dissolved dilute to 120 ml. Add sufficient KOH soln (1+1) to discharge the I color.

(b) *Sodium sulfite soln.*—Dissolve 15 g of Na<sub>2</sub>SO<sub>3</sub> in H<sub>2</sub>O and dilute to 100 ml.

163

## PREPARATION OF ALCOHOLIC EXTRACT

To a 1 g sample in a 50 ml volumetric flask, add ca 35 ml of alcohol; boil gently ca 20 min., rotating flask occasionally; cool, and make up to volume with alcohol. Mix thoroly, filter thru dry paper (covering funnel with watch-glass to avoid evaporation), pipet a number of aliquots of 10 ml each (or sufficient to contain ca 0.2 g of phenolphthalein) into 250 ml beakers, and evaporate to dryness on steam bath.

164

## DETERMINATION

Take up residue in alkali by moistening with ca 1 ml of KOH soln (1+1) and add a little H<sub>2</sub>O. When residue is completely in soln add a piece of ice (ca 40 g), 4–4.5 ml of the prepared I reagent, and HCl from a buret, dropwise, using a stirring rod (beaker is not rotated) to complete precipitation. If sufficient I has been added, the precipitate as well as the supernatant liquid will be brown; if not, add more I to insure excess, and then the KOH soln dropwise, with stirring, to dissolve the precipitate completely and consume all excess I. (This soln should be blue or blue-purple.) Repeat process of precipitation with strong acid and resolution with strong alkali 3 or 4 times with small quantities of the reagents, adding small pieces of ice if necessary to keep the soln cold. In the acid condition there should be a brown precipitate resembling a periodide, and the supernatant liquid should be colored brown by the excess I. (The alkaline soln should be clear blue or purple-blue, and no precipitate should be present.) Then add 1 or 2 ml of the Na<sub>2</sub>SO<sub>3</sub> soln to the alkaline soln and filter the ice-cold mixture thru a Gooch crucible into a tall 250 ml beaker, using a bell jar arrangement and washing several times with H<sub>2</sub>O. Acidify the filtrate with HCl, using a few ml in excess, and heat on steam bath 20–30 min. Collect coagulated precipitate in weighed Gooch crucible, wash a few times with H<sub>2</sub>O, and when sucked fairly dry wash several times with petroleum benzin. Dry precipitate in oven (120–140°) to constant weight. Weight of precipitate × 0.3872 = weight of phenolphthalein.

PYRIDIDIUM<sup>20</sup>—TENTATIVE

165

## REAGENTS

(a) *Standard titanium trichloride soln.*—Prepare as directed in XXI, 35, and standardize as directed in XXI, 36, Method II.

(b) *Light green S F yellowish soln.*—Dissolve 1 g in H<sub>2</sub>O and dilute to 1000 ml.

166

## PREPARATION OF SAMPLE

*Solutions.*—To a volume containing ca 0.1 g of pyridium, add 10 ml of 0.1 N HCl and dilute to 100 ml.

*Tablets and jelly.*—Weigh a quantity of sample (powdered in case of tablets) equivalent to ca 0.1 g of pyridium, add 10 ml of 0.1 N HCl, and dilute to 100 ml.

*Ointments.*—Weigh in a 100 ml beaker a portion of sample equivalent to ca 0.1 g of pyridium, stir with ether until ointment base is dissolved, and wash into a



separator with ether and  $\text{H}_2\text{O}$ . Shake thoroly and draw off aqueous layer into a second separator containing 25 ml of ether. Shake, and draw off aqueous layer into a third separator containing 25 ml of ether. Shake, and transfer aqueous layer to a 250 ml beaker. Wash ether layers with alternate 10 ml portions of  $\text{HCl}$  (1+1) and  $\text{H}_2\text{O}$  until no more color is removed, passing each portion of  $\text{HCl}$  or  $\text{H}_2\text{O}$  successively thru the three separators and finally into beaker. Nearly neutralize combined acid extracts with  $\text{NH}_4\text{OH}$ , cool, wash into a separator, make ammoniacal, and extract with 25 ml portions of  $\text{CHCl}_3$  until no more color is removed, filtering the  $\text{CHCl}_3$  thru a pledget of cotton in stem of separator. Evaporate combined  $\text{CHCl}_3$  extracts just to dryness, take up in 10 ml of 0.1  $N$   $\text{HCl}$  and dilute to 100 ml.

167

## DETERMINATION

Heat soln to boiling, add 15 g of  $\text{Na}$  acid tartrate, and boil 2 min. Add 10 ml of the light green  $SF$  yellowish soln and titrate hot with the standard  $\text{TiCl}_3$  soln in a current of  $\text{CO}_2$ . The end point is the change from green to pale yellow. Run a blank titration with 10 ml of 0.1  $N$   $\text{HCl}$ , 90 ml of  $\text{H}_2\text{O}$ , 15 g of  $\text{Na}$  acid tartrate, and 10 ml of the light green  $SF$  yellowish soln, and subtract from the volume of  $\text{TiCl}_3$  previously found. 1 ml of 0.1  $N$   $\text{TiCl}_3 = 0.00624$  g of pyridium ( $\text{C}_{11}\text{H}_{11}\text{N}_5 \cdot \text{HCl}$ ).

168

SULFANILAMIDE<sup>81</sup>—TENTATIVE

Place on a 9 cm folded filter paper in a funnel a portion of sample containing ca 0.5 g of sulfanilamide. Wash soluble portion with a fine stream of acetone into a 250 ml flask, using total of ca 25 ml of acetone. Test for complete extraction by evaporating a small portion of washings. Immerse flask in a  $\text{H}_2\text{O}$  bath at ca  $70^\circ$  until acetone has been evaporated and its odor is no longer perceptible. Remove from bath and add 10–12 ml of 75% (by volume)  $\text{H}_2\text{SO}_4$ . Connect flask to reflux condenser with water jacket, add a few glass beads, and boil soln slowly 30 min. Wash down condenser with  $\text{H}_2\text{O}$ , make liquid in flask to ca 100 ml with  $\text{H}_2\text{O}$ , add an excess of 50% alkali, distil, and collect the ammonia in distillate in an excess of 0.1  $N$   $\text{H}_2\text{SO}_4$ . Titrate excess acid with 0.1  $N$   $\text{NaOH}$ , using methyl red indicator. 1 ml of 0.1  $N$   $\text{H}_2\text{SO}_4 = 0.01722$  g of  $(\text{NH}_2)_2\text{C}_6\text{H}_4\text{SO}_2$ .

## SANTONIN IN MIXTURES

169

*Langer's Method (Modified)*<sup>82</sup>—Official

Weigh out a sample equivalent to ca 0.15 g of santonin, and extract with 10, 10, 10, 5, and 5 ml portions of petroleum benzin saturated with santonin. (If sample is fat-free this step may be omitted.) Filter each portion of solvent with aid of suction to complete dryness thru a Gooch crucible provided with asbestos mat before following with another portion of fresh solvent. Extract residue in soln flask and crucible with 15, 10, 5, and 5 ml of hot benzene, filtering each portion as before. Evaporate the benzene extract in a tared flask and dry residue to constant weight at  $100^\circ$ . Weight of santonin in flask = weight of santonin in sample.

*Dinitrophenylhydrazine Method*<sup>83</sup>—Official

170

## REAGENT

*Dinitrophenylhydrazine sulfate soln.*—Dissolve 1 g of 2,4 dinitrophenylhydrazine in a mixture of 90 ml of  $\text{H}_2\text{O}$  and 10 ml of  $\text{H}_2\text{SO}_4$  by warming, cool, and filter.

171

## DETERMINATION

Weigh 2.5 g of ground sample into a Gooch crucible and wash with ca 100 ml of petroleum benzin saturated with santonin. Discard washings. Extract with ca 100 ml of hot benzene, collecting filtrate in a beaker. Evaporate to dryness, warm residue

with alcohol until dissolved, transfer to a 100 ml volumetric flask, cool, make to volume at 20° with alcohol, and filter if necessary. To 25 ml of the soln add 50 ml of the dinitrophenylhydrazine soln and allow to stand 48 hours in dark place. Collect precipitate in a Gooch crucible and wash it with dilute alcohol (1+2), using a total volume of ca 150 ml. Dry residue 1 hour at 100°, cool, and weigh. Weight of precipitate  $\times 0.5775$  = weight of santonin.

172

SANTONIN IN SANTONICA (LEVANT WORM SEED)<sup>34</sup>—TENTATIVE

Extract 3 g of ground sample with benzene in a Soxhlet apparatus or an automatic percolator (Fig. 55) for 3 hours. Wash extract into a separator with a little benzene, add more benzene if necessary to make a total volume of ca 100 ml, and shake vigorously for 5 min. with 35 ml of 8%  $\text{Na}_2\text{CO}_3$  soln. Allow mixture to separate completely and transfer aqueous layer to a second separator. Wash the benzene once with 10 ml of  $\text{H}_2\text{O}$  and add washing to second separator. Shake combined aqueous extracts with 10 ml of benzene, discard aqueous layer, wash benzene with 5 ml of  $\text{H}_2\text{O}$ , and combine with benzene in first separator. Filter the benzene soln thru cotton and evaporate filtrate to dryness. Warm residue with 5 ml of alcohol until mass is disintegrated, and add 60 ml of saturated aqueous soln of  $\text{Ba}(\text{OH})_2$  while stirring. Heat mixture to boiling, place on steam bath 10 min., filter into a separator, and wash filter and beaker with two 10 ml portions of hot  $\text{Ba}(\text{OH})_2$  soln. Add 6 ml of  $\text{HCl}$  (2+1) to filtrate, cool, and extract with 25, 15, 10, 10, and 5 ml portions of  $\text{CHCl}_3$ , filtering thru a pledget of cotton in stem of funnel, and evaporate filtrate to dryness. Dissolve residue in 25 ml of alcohol by warming, mix soln with 50 ml of dinitrophenylhydrazine sulfate soln, 170, and proceed as directed in 171, beginning "allow to stand 48 hours."

ARSENIC IN IRON-ARSENIC TABLETS<sup>35</sup>—OFFICIAL

173

## REAGENT

*Standard soln of potassium bromate (or of iodine).*—Standardize against pure  $\text{As}_2\text{O}_3$ . (The strength of this soln is a matter of choice. 0.5625 g of  $\text{KBrO}_3$  dissolved in  $\text{H}_2\text{O}$  and diluted to 1 liter gives a soln that is 0.02021 *N*, 1 ml of which = 0.001 g of  $\text{As}_2\text{O}_3$ .)

174

## APPARATUS

Use either the Ramberg-Sjöström arsenic flask, which consists of a 300 ml Kjeldahl flask provided with a specially shaped outlet tube connected with the flask by means of a ground joint (*A*, Fig. 60), or a 300 ml Kjeldahl flask provided with an outlet tube, the internal diameter of the main part of which is ca 13 mm and that of the contracted tip ca 5 mm, connected with the flask by means of a rubber stopper (*B*).

175

## DETERMINATION

Weigh and place in the flask 5–10 tablets or pills, add 10–15 ml of  $\text{H}_2\text{O}$ , and allow to soak 30 min. Add, small portions at a time, 20 ml of fuming  $\text{HNO}_3$ , cooling if necessary to prevent loss by frothing. When the reaction has ceased, add carefully and in small portions at a time 25–28 ml of  $\text{H}_2\text{SO}_4$ . Place flask in inclined position on asbestos mat and heat over small flame. As soon as greater part of the  $\text{HNO}_3$  has been driven off, and while still heating, drop in 8 ml of fuming  $\text{HNO}_3$  thru a suitably placed separator and heat over larger flame until  $\text{SO}_3$  is evolved. If after cooling the precipitated sulfates are not colorless or pale yellow and are not free

from gray or black particles, heat contents of flask further with an additional 10 ml of fuming  $\text{HNO}_3$ . (It is essential that all organic matter be destroyed.) To cooled mixture add 30 ml of a saturated soln of  $\text{NH}_4$  oxalate; heat until fumes of  $\text{SO}_3$  are evolved and, to insure complete destruction of the oxalic acid, for 10 min. thereafter over low flame; cool; and add while gently swirling flask 20 ml of  $\text{H}_2\text{O}$ . Dry neck of flask over small flame and add 30 g of  $\text{NaCl}$ , 5 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (or 1 g of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$ ), 1 g of  $\text{NaBr}$ , and 25 ml of  $\text{HCl}$ . Mix contents of flask and connect delivery tube. If the Ramberg-Sjöström apparatus is used, moisten the ground-glass joint with a drop of  $\text{H}_2\text{SO}_4$ . Fix flask in inclined position with tip of outlet tube ca 1 cm under surface of 150 ml of  $\text{H}_2\text{O}$  in an Erlenmeyer flask surrounded by ice or by cold  $\text{H}_2\text{O}$ . Distil at such a rate that bend at top of tube becomes warm in 4 min. and lower end in ca 8 min. from time heat is applied. Discontinue distillation at end of 10 min., but before removing flame lift distillation flask until tip of outlet tube is

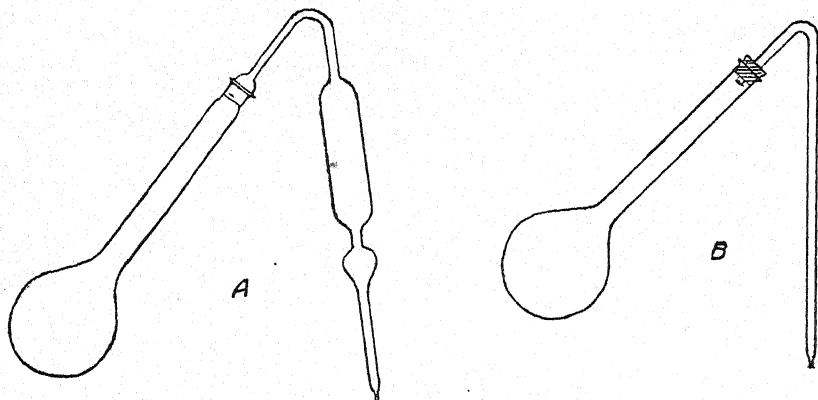


FIG. 60.—APPARATUS FOR THE DETERMINATION OF ARSENIC IN IRON-ARSENIC TABLETS

above the  $\text{H}_2\text{O}$  in the receiving flask. Let outlet tube drain, remove receiver, and either titrate with the standard soln of  $\text{KBrO}_3$ , using 2 drops of methyl orange indicator (red color of indicator at end point may fade slowly, but color should persist at least 1 min. upon addition of another drop of indicator); or nearly neutralize with  $\text{NaOH}$ , add 4–5 g of  $\text{NaHCO}_3$ , and titrate with the standard soln of  $\text{I}$ , using starch indicator, VI, 3(e).

176

#### ARSENIC IN SODIUM CACODYLATE<sup>86</sup>—OFFICIAL

Transfer 0.2 g of sample, accurately weighed, to a Kjeldahl flask. Add 10 g of  $\text{K}_2\text{SO}_4$ , 0.3 g of starch, and 20 ml of  $\text{H}_2\text{SO}_4$ . Digest over low flame until frothing has ceased. Continue digestion 4 hours or until mixture is colorless. Cool, dilute with  $\text{H}_2\text{O}$ , and transfer to a 500 ml Erlenmeyer flask. Add  $\text{NaOH}$  soln (1+1) slowly until alkaline to litmus paper and acidify with  $\text{H}_2\text{SO}_4$ . Place flask in  $\text{H}_2\text{O}$  until thoroly cooled, add 5 g of  $\text{NaHCO}_3$ , and titrate with 0.1 *N*  $\text{I}$  soln. Conduct a blank, using same quantities of reagents. 1 ml of 0.1 *N*  $\text{I}$  soln = 0.00375 g of As, or 0.008 g of anhydrous  $\text{Na}(\text{CH}_3)_2\text{AsO}_2$ .

177

#### ARSENIC IN IRON METHYLARSENATE<sup>87</sup>—OFFICIAL

Transfer a suitable quantity of sample (0.2 g, if practicable) to a Kjeldahl flask. Add 10 g of  $\text{K}_2\text{SO}_4$ , 0.3 g of starch, and 20 ml of  $\text{H}_2\text{SO}_4$ . Digest over low heat until frothing has ceased and continue digestion over a slightly higher flame until mixture

is colorless. Cool, and add 20 ml of  $\text{H}_2\text{O}$ . Dry neck of flask over small flame; cool contents; and add 30 g of  $\text{NaCl}$ , 5 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 1 g of  $\text{NaBr}$ , and 25 ml of  $\text{HCl}$ . Distil as directed under 175. Conduct a blank, using same quantities of reagents.

BISMUTH COMPOUNDS IN TABLETS<sup>88</sup>—TENTATIVE

178

(Lead absent.)

Thoroughly mix sample and weigh 0.5 g into a 500 ml Kjeldahl flask. Ignite gently over small flame, using a wire gauze under flask, and increase heat towards end. Allow to cool, add 15–20 ml of  $\text{HNO}_3$ , evaporate to dryness, and ignite as before until yellow or orange  $\text{Bi}_2\text{O}_3$  is formed. Cool residue, and dissolve in 10–15 ml of warm  $\text{HNO}_3$ , using a few ml of 3%  $\text{H}_2\text{O}_2$  if there is difficulty in obtaining a soln. Boil off excess  $\text{H}_2\text{O}_2$  and wash into a 400 ml beaker with  $\text{H}_2\text{O}$ , rinsing flask well. Dilute to ca 200 ml, make just neutral to litmus with  $\text{NH}_4\text{OH}$ , and add 5 ml of  $\text{HCl}$ . Precipitate with  $\text{H}_2\text{S}$  completely.

Transfer precipitate to a filter paper and wash once with  $\text{HCl}$  (5+200) and then several times with  $\text{H}_2\text{O}$ . Dissolve the precipitate of  $\text{Bi}_2\text{S}_3$  on a filter with hot  $\text{HNO}_3$  (1+2). A small residue of S (and  $\text{HgS}$  if  $\text{Hg}$  salts are present) usually remains. Neutralize filtrate with 10%  $\text{NH}_4\text{OH}$  and precipitate with 25 ml of a 20% soln of ammonium carbonate. Concentrate to ca 150 ml (by boiling, if desired) and allow to stand on steam bath 1–2 hours. Collect precipitate in a previously ignited weighed Gooch crucible, wash with small quantity of  $\text{H}_2\text{O}$ , dry, ignite in muffle at red heat, and weigh as  $\text{Bi}_2\text{O}_3$ .

179

CALCIUM GLUCONATE<sup>89</sup>—TENTATIVE

(Applicable to preparations the aqueous solns of which are neutral and do not contain salts of other optically active hydroxy acids.)

Weigh two 0.5 g portions of  $\text{Ca}$  gluconate or two 1 g portions of powdered tablets containing 50% or less of the salt. If chocolate or a fatty base is present, wash samples several times on hardened filter with absolute ether and warm residue until ether is driven off. Transfer each portion to a separate 25 ml volumetric flask, add 15 ml of  $\text{H}_2\text{O}$ , and warm until the calcium salt is dissolved (there will be an undissolved residue in the case of samples containing cocoa). Cool mixture to room temp. To one flask (No. 1) add 3.5 g of finely pulverized uranyl acetate, stopper, and place mixture in shaking machine for 1 hour (if agitation is not sufficiently vigorous, more than 1 hour's shaking may be required). Allow other flask (No. 2) to stand. If sample contains chocolate, add a little alumina cream, XXXIV, 19(b), to each flask. Cool to 20°, make up contents of flask No. 1 to volume with uranyl acetate soln (10 g shaken with 95 ml of  $\text{H}_2\text{O}$  until saturated, then filtered), and flask No. 2 with  $\text{H}_2\text{O}$ . Filter, and polarize each soln in a 200 mm tube, using a 50 mm tube containing a 1.8% soln of  $\text{K}_2\text{Cr}_2\text{O}_7$  as a light filter. If soln is too dark to read in the 200 mm tube, make reading in a 100 mm tube and multiply result by 2. If  $A$  = rotation in °V. of soln No. 2 and  $B$  = the rotation of soln No. 1, with 1 g samples the percentage of  $\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2 = 4.34 (B - A)$ , and with 0.5 g samples the percentage of  $\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2 = 8.52 (B - A)$ .

## HYPOPHOSPHITES IN SIRUPS—TENTATIVE

(Applicable in absence of phosphates; if phosphates are present, make suitable correction.)

180

Method I<sup>90</sup>

(a) *Total hypophosphites*.—Place 25 ml of sample in a 100 ml volumetric flask, dilute to mark, and mix thoroughly. Pipet a 10 ml aliquot into a suitable flask. Add 25

ml of  $\text{HNO}_3$ , and boil on hot plate until volume is reduced to 2–3 ml; add 10 ml of  $\text{HNO}_3$ , and boil until volume is again reduced to 2–3 ml. Cool, and add 20 ml of  $\text{H}_2\text{O}$ . Add  $\text{NH}_4\text{OH}$  in slight excess, and barely dissolve precipitate formed with a few drops of  $\text{HNO}_3$ , stirring vigorously. To the hot soln add 70 ml of molybdate soln, II, 7(a), for each 0.1 g of  $\text{P}_2\text{O}_5$  present. Digest at ca  $65^\circ$  for 1 hour, and test for complete precipitation by addition of more reagent to clear supernatant liquid. Filter, and wash with  $\text{NH}_4\text{NO}_3$  soln, II, 7(b).

Dissolve precipitate on filter with  $\text{NH}_4\text{OH}$  (1+1) and hot  $\text{H}_2\text{O}$  and wash into a beaker to volume of not more than 100 ml. Nearly neutralize with  $\text{HCl}$ , using litmus paper as indicator. Cool, and from a buret add slowly (ca 1 drop per second, stirring vigorously) 15 ml of magnesia mixture, II, 7(c), for each 0.1 g of  $\text{P}_2\text{O}_5$  present. After 15 min. add 12 ml of  $\text{NH}_4\text{OH}$  and allow to stand overnight. Filter, and wash precipitate with dilute  $\text{NH}_4\text{OH}$ , II, 7(d), until washings are practically free from chlorides. Dry, burn first at low heat, and ignite to constant weight, preferably in electric furnace, at  $950\text{--}1000^\circ$ . Cool, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ .  $\text{Mg}_2\text{P}_2\text{O}_7 \times 0.6379 = \text{P}_2\text{O}_5$ .

(b) *Calcium*.—Using prepared soln (a), pipet a 20 ml aliquot into a 400 ml beaker and dilute to 100 ml. Add 2 ml of  $\text{HCl}$ , 15 ml of 10%  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln, and a slight excess of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln. Heat to boiling and allow precipitate to settle at temp. just below boiling. Filter hot, wash with 1%  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln, dry, moisten with  $\text{H}_2\text{SO}_4$ , ignite gently, and weigh residue as  $\text{CaSO}_4$ .  $\text{CaSO}_4 \times 0.2944 = \text{Ca}$ .

#### Method II<sup>91</sup>

(Not applicable in presence of other reducing agents or of phenolic compounds.)

181

#### REAGENTS

- (a) *Bromide-bromate soln*.—See 26(c).
- (b) *Sodium thiosulfate*.—0.1 N. See 3(b).
- (c) *Potassium iodide*.—20 g per 100 ml.

182

#### DETERMINATION

Transfer 50 ml of the sirup, measured in a 50 ml volumetric flask, to a 250 ml volumetric flask. Wash the 50 ml flask with several portions of  $\text{H}_2\text{O}$ , adding washings to 250 ml flask, finally making up to the mark with  $\text{H}_2\text{O}$ , and mixing well. (This procedure is followed in the case of the sirup of  $\text{NH}_4\text{H}_2\text{PO}_2$ . For sirups containing larger quantities of hypophosphites the original 50 ml may be diluted to 500 ml in a volumetric flask.) Transfer a 50 ml aliquot to a 250 ml volumetric flask and make up to mark with  $\text{H}_2\text{O}$ , again mixing well. Of this soln, transfer a 50 ml aliquot to a glass-stoppered 250 ml flask, add 50 ml of the bromide-bromate soln and 20 ml of 10%  $\text{H}_2\text{SO}_4$ ; stopper, shake well, and let stand 2 hours. Add 10 ml of the  $\text{KI}$  soln, shake flask, and titrate liberated  $\text{I}$  with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln until a straw color appears; add 2 ml of starch soln, VI, 3(e), and titrate until the soln becomes colorless. Conduct a blank determination in same way. 1 ml of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3 = 0.00165$  g of  $\text{H}_3\text{PO}_2$ ; 1 ml of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3 = 0.00208$  g of  $\text{NH}_4\text{H}_2\text{PO}_2$ .

183

#### IODINE<sup>92</sup>—TENTATIVE

Transfer a quantity of sample that contains not more than 0.1 g of the iodide (0.05 g is ample) to a crucible, preferably nickel, and add 2 or 3 g of solid  $\text{KOH}$ . If sample is a solid, add 10–15 ml of alcohol before adding the  $\text{KOH}$ . Dry, and char thoroly. (Use as low a temp. as possible in order to prevent loss of iodide, in no event more than dull redness.) Extract charred mass with hot  $\text{H}_2\text{O}$ , filter into an Erlenmeyer flask, and wash well with hot  $\text{H}_2\text{O}$ .

Neutralize filtrate with  $\text{H}_2\text{SO}_4$  (1+1), make alkaline again, with 4%  $\text{NaOH}$  soln, and add 1 ml in excess. Heat to boiling and add saturated  $\text{KMnO}_4$  soln slowly until permanganate color remains after several minutes' boiling. Then add ca 0.5 ml in excess, continue boiling ca 5 min., and allow to cool. Add a few ml of alcohol and set on steam bath. (Permanganate color should be bleached; if it is not, add a little more alcohol.) When precipitate has settled, filter and wash with hot  $\text{H}_2\text{O}$ . After cooling, add 1-2 g of  $\text{KI}$  (crystals), acidify with  $\text{HCl}$ , and titrate with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  soln. 1 ml of 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  = 0.002768 g of  $\text{KI}$ , 0.002498 g of  $\text{NaI}$ , or 0.002116 g of  $\text{I}$ .

FREE IODINE IN IODINE OINTMENT<sup>93</sup>—TENTATIVE

184

## REAGENT

*Potassium arsenite soln.*—Dissolve 4.948 g of  $\text{As}_2\text{O}_3$  in a soln of  $\text{NaOH}$  (4 g + 4 ml of  $\text{H}_2\text{O}$ ), add 100 ml of a saturated aqueous soln of  $\text{KHCO}_3$ , and make to 1000 ml with  $\text{H}_2\text{O}$ .

185

## DETERMINATION

Weigh accurately 4-5 g of the ointment in an iodine flask, stopper, and heat on water bath until sample is just fluid. Add 30 ml of  $\text{CHCl}_3$  and shake with rotary motion until base is dissolved. Add 30 ml of  $\text{H}_2\text{O}$  and titrate immediately with the  $\text{KAsO}_2$  soln, using starch as indicator. 1 ml of 0.1  $N$   $\text{KAsO}_2$  = 0.01269 g of  $\text{I}$ .

IODOFORM<sup>94</sup>—OFFICIAL

186

## REAGENTS

(a) *Ammonium thiocyanate soln.*—0.05  $N$ . Standardize against 0.1  $N$   $\text{AgNO}_3$  soln, using an equal volume of alcohol and 3 ml of  $\text{FeNH}_4(\text{SO}_4)_2$  soln as indicator.

(b) *Ferric ammonium sulfate indicator.*—Dissolve 8 g of  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .

187

## DETERMINATION

Weigh accurately ca 0.25 g of  $\text{CHI}_3$  and transfer quantitatively to a 200 ml Erlenmeyer flask. Add 40 ml of alcohol, swirl gently until the  $\text{CHI}_3$  is dissolved, filter if necessary, and immediately add 40 ml of 0.1  $N$   $\text{AgNO}_3$  and 10 ml of  $\text{HNO}_3$ . Swirl gently ca 5 min., allow to stand at room temp. 2-3 hours, and then swirl occasionally as an aid in flocculating the  $\text{AgI}$ . Titrate excess 0.1  $N$   $\text{AgNO}_3$  with 0.05  $N$   $\text{NH}_4\text{CNS}$ , using 3 ml of the  $\text{FeNH}_4(\text{SO}_4)_2$  indicator. 1 ml of 0.1  $N$   $\text{AgNO}_3$  = 0.01313 g of  $\text{CHI}_3$ . Or, filter, collecting the  $\text{AgI}$  on a dried and accurately weighed Gooch crucible, wash with  $\text{H}_2\text{O}$  and finally with alcohol, and dry to constant weight at ca  $125^\circ$ . 1 g of  $\text{AgI}$  = 0.5590 g of  $\text{CHI}_3$ .

188

IODOFORM OINTMENT<sup>95</sup>—OFFICIAL

Transfer ca 2.5 g of sample to a tared 50 ml beaker and weigh. Add 5 ml of  $\text{CHCl}_3$ , stir gently with glass rod, and transfer bulk of undissolved ointment and the  $\text{CHCl}_3$  soln to a 250 ml flask having a ground-glass stopper. Add 5 ml of  $\text{CHCl}_3$  to ointment remaining in beaker and stir until dissolved. Add the soln to contents of flask and finally wash beaker three times, using not more than 5 ml of  $\text{CHCl}_3$  each time, and add washings to contents of flask. Or, weigh sample in a small, tared glass capsule, drop capsule with contents into a 250 ml flask having a ground-glass stopper, and add not more than 20 ml of  $\text{CHCl}_3$ . (Use glass capsule only in volumetric determination.) Swirl gently until all ointment is dissolved. Add 40 ml of alcoholic 0.1  $N$

$\text{AgNO}_3$  soln and swirl to wash down any  $\text{CHI}_3$  that may adhere to sides of flask. Slowly add 10 ml of  $\text{HNO}_3$  and allow to stand at room temp. ca 18 hours. Titrate excess of alcoholic 0.1  $N$   $\text{AgNO}_3$  soln with 0.05 ammonium thiocyanate soln, 186(a), using 3 ml of  $\text{FeNH}_4(\text{SO}_4)_2$  indicator, 186(b), shaking mixture vigorously near end of titration. 1 ml of 0.1  $N$   $\text{AgNO}_3 = 0.01313$  g of  $\text{CHI}_3$ .

For gravimetric determination use an ordinary Erlenmeyer flask in place of the flask having a ground-glass stopper. Weigh the ointment base into a 100 ml beaker and add  $\text{CHCl}_3$ . When the ointment base has dissolved, filter thru a Gooch crucible, using suction. Wash beaker and crucible once with alcohol. Wash crucible several times with  $\text{CHCl}_3$  without using suction. Collect filtrate in an Erlenmeyer flask and add 40 ml of 0.1  $N$   $\text{AgNO}_3$  soln and 10 ml of  $\text{HNO}_3$  in small portions. Allow mixture to stand 18 hours. Collect the  $\text{AgI}$  on a weighed Gooch crucible, using suction. Wash with  $\text{H}_2\text{O}$  and then with alcohol. Finally wash repeatedly with  $\text{CHCl}_3$  without suction. Dry the Gooch crucible and contents at ca  $125^\circ$  to constant weight. 1 g of  $\text{AgI} = 0.5590$  g of  $\text{CHI}_3$ .

189

IODOFORM GAUZE<sup>96</sup>—OFFICIAL

Weigh in a tared weighing bottle with ground-glass stopper a sample of  $\text{CHI}_3$  gauze containing ca 1 g of  $\text{CHI}_3$ . ( $\text{CHI}_3$  gauze is usually moist and loses weight rapidly when exposed to air.) Transfer to a 150 ml beaker, add ca 75 ml of alcohol, and stir until  $\text{CHI}_3$  is dissolved. Filter into a 200 ml volumetric flask, draining the alcoholic soln with the aid of pressure upon the gauze. Wash 4 or 5 times, using 25 ml of alcohol each time, filter washings, and finally make up to volume with alcohol. Pipet a 40 ml aliquot into a 200 ml Erlenmeyer flask and immediately add 40 ml of 0.1  $N$   $\text{AgNO}_3$  and 10 ml of  $\text{HNO}_3$ . Proceed as directed under 188, beginning "allow to stand at room temp."

MERCUROUS CHLORIDE (CALOMEL) IN TABLETS<sup>96</sup>—OFFICIAL

190

## REAGENT

*Standard iodine soln.*—0.1  $N$ . Dissolve ca 14 g of I in a soln containing 18 g of KI in 100 ml of  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize this soln against standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 3(b).

191

## DETERMINATION

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of well-mixed sample to represent 0.19–0.26 g (3–4 grains) of  $\text{HgCl}_2$ . Transfer to a 200 ml glass-stoppered Erlenmeyer flask, add ca 50 ml of  $\text{H}_2\text{O}$ , acidify with acetic acid, and after soluble fillers have dissolved decant with aid of suction thru a tightly packed asbestos mat placed on plate of a Caldwell crucible. Wash once with  $\text{H}_2\text{O}$  by decantation, then successively with alcohol and ether. Transfer removable plate holding mat and insoluble material to original flask, washing into flask any insoluble material adhering to sides of crucible. Add 2.5 g of KI, 10 ml of  $\text{H}_2\text{O}$ , and then 30 ml of the standard I soln. Allow mixture to stand, with frequent and fairly vigorous agitation, ca 1.5 hours, or until soln of calomel is complete. Titrate with the standard thiosulfate soln, 3(b), and add ca 1 ml in excess. Titrate back with the standard I soln, using starch indicator, VI, 3(e), until a permanent blue color is obtained. 1 ml of 0.1  $N$  I soln = 0.02361 g of  $\text{HgCl}_2$ .

192

CALOMEL IN CALOMEL OINTMENTS<sup>97</sup>—TENTATIVE

Weigh accurately ca 1 g of ointment, transfer to a 250 ml glass-stoppered Erlenmeyer flask, and treat with ca 50 ml of  $\text{CHCl}_3$ . When base is dissolved, decant thru

a dry closely packed asbestos mat in a Caldwell crucible, using light suction. Wash flask and contents several times with 20 or 30 ml portions of  $\text{CHCl}_3$ , decanting thru crucible. Allow any residual  $\text{CHCl}_3$  in flask to evaporate and transfer asbestos mat and contents to flask, wiping sides of crucible and mouth of flask with a damp piece of filter paper and adding it to contents of flask. Add 2.5 g of KI and 50 ml of 0.1 N I soln, 190, stopper, and mix contents well. Allow flask to stand ca 1.5 hours or until soln of calomel is complete, agitating it frequently and fairly vigorously. Titrate with 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ , adding 1 or 2 ml in excess and using starch as indicator, VI, 3(e). When all traces of I have disappeared, titrate back with the standard I soln until a blue color is obtained. 1 ml of 0.1 N I = 0.02361 g of HgCl.

193

MERCUROUS IODIDE IN TABLETS<sup>98</sup>—TENTATIVE

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of well-mixed sample to represent 0.19–0.26 g (3–4 grains) of HgI. Transfer sample to a 200 ml glass-stoppered flask, add ca 50 ml of  $\text{H}_2\text{O}$ , acidify with acetic acid, and after soluble fillers have dissolved, decant with aid of suction thru a tightly packed asbestos mat placed on plate of a Caldwell crucible. Wash once with  $\text{H}_2\text{O}$  by decantation, then successively with alcohol and ether. Transfer removable plate holding mat and insoluble material to original flask, washing into flask any insoluble materials adhering to sides of crucible. Add 2.5 g of KI and 30 ml of I soln, 190. Allow mixture to stand, with frequent and fairly vigorous agitation, ca 1.5 hours, or until soln of HgI is complete. Titrate with standard thiosulfate soln, 3(b), and add 1 or 2 ml in excess. When all traces of I have disappeared, titrate back with standard I, using starch indicator. 1 ml of 0.1 N I soln = 0.03275 g of HgI.

NOTE: Some commercial tablets are difficult to filter thru the asbestos mat without loss of HgI. A few drops of alumina cream, XXXIV, 19(b), washed free from  $\text{NH}_3$ , placed on the mat before filtration is started, satisfactorily prevents loss, tho it retards filtration.

MERCUROCHROME<sup>99</sup>—TENTATIVE

194

*Tests for Purity*

(a) Acidify a portion of the mercurochrome soln with 10%  $\text{H}_2\text{SO}_4$  and filter off precipitate. The filtrate is colored only slightly yellow.

(b) Pass  $\text{H}_2\text{S}$  into a portion of filtrate. No precipitate or coloring occurs.

(c) Add a few ml of 10%  $\text{HNO}_3$  to another portion of the filtrate and add  $\text{AgNO}_3$  soln. No precipitate forms.

195

*Total Solids in Solution<sup>99</sup>—Tentative*

Pipet 10 ml of the mercurochrome soln into a tared, extra-wide-form weighing bottle and evaporate to dryness on steam bath. Allow to dry overnight in the open bottle in a desiccator containing  $\text{H}_2\text{SO}_4$ . Weigh.

196

*Determination of Mercury<sup>100</sup>—Tentative*

Pipet 10 ml of an approximately 2% soln of the mercurochrome into a 500 ml tall form beaker and evaporate to dryness on steam bath (or weigh accurately ca 0.2 g of the powder). Dissolve residue in 4 ml of  $\text{H}_2\text{O}$  and add slowly, with constant mixing, 10 ml of  $\text{H}_2\text{SO}_4$ . Incline beaker and add cautiously small portions of  $\text{KMnO}_4$  (finely pulverized), mixing after each addition, until considerable excess has been added, as indicated by deep purple color of mixture. Allow to stand 30 min., occasionally mixing, at end of which time mixture should still retain its purple color.



Add 100 ml of  $H_2O$  and mix thoroly. Add small portions of oxalic acid (finely pulverized), mixing after each addition, until the soln is clear. Filter thru a small filter into a 400 ml beaker, wash original beaker and filter until filtrate measures ca 200 ml, and pass  $H_2S$  thru the soln 20 min. Warm on steam bath until precipitate of  $HgS$  settles quickly after stirring, and again pass  $H_2S$  thru the warm soln 5 min. Filter the soln immediately into a weighed Gooch crucible, and wash precipitate on filter well with  $H_2O$ , three times with the alcohol, and then with 4 or 5 portions of  $CCl_4$  or  $CS_2$ , allowing liquid to run thru crucible without suction, and finally wash with ether. Dry precipitate to constant weight at  $100^\circ$ , and weigh as  $HgS$ . Test dried precipitate qualitatively for  $Hg$  and other heavy metals. If any difficulty is experienced by slow filtration during the washing with  $H_2O$ , allow precipitate to drain and wash once with alcohol, then continue as directed.  $HgS \times 0.8622 = Hg$ .

197

MERCURY IN OINTMENT OF MERCURIC NITRATE<sup>101</sup>—TENTATIVE

Transfer to a 200–300 ml Erlenmeyer flask 3–5 g of sample accurately weighed, using a glass or bone spatula. Add 40 ml of  $HNO_3$  (1+1) and a few glass beads and insert a short-stemmed funnel into neck of flask. Boil gently 1–1.5 hours on hot plate or over low flame. With latter use piece of asbestos having a circular hole under an asbestos wire gauze. Add 30 ml of  $H_2O$ , using a part to wash funnel. Cool sufficiently to cause unconsumed fat to form a hard cake (ca  $20^\circ$  or below). Filter thru an 11 cm filter into a 200 ml volumetric flask. Wash the fat, flask, and filter, using ca 100 ml of 1%  $HNO_3$ . Make to volume and mix well. Reserve fat for test for complete extraction as directed below.

Transfer a 100 ml aliquot to a 500 ml Erlenmeyer flask. Add 7 ml of  $HNO_3$ , 5 ml of  $H_2SO_4$ , and 2 g of powdered permanganate, and rotate to dissolve. Heat just to boiling over low flame or on hot plate. Boil gently 45 min., maintaining an excess of permanganate, indicated by dark purple color. (An excess is essential.) When adding permanganate to boiling liquid use smaller portions (ca 0.5 g or less) to avoid loss due to frothing.

**CAUTION:** Use of greater excess of permanganate than is necessary is not objectionable, but it will require proportionately more of the peroxide to remove it and the  $MnO_2$  at end of digestion. Usually ca 10 g is required. The rate of consumption and total permanganate consumed seem to vary with temp., organic matter present, and period of heating. The large amount of  $MnO_2$  formed may lead to the wrong conclusion concerning the color indicative of an excess of permanganate. Frequent examination of the soln is necessary. The observation of this color is aided when the analyst looks thru the supernatant liquid toward a white background while holding the container in an inclined position.

Remove excess permanganate and dissolve  $MnO_2$  by adding  $H_2O_2$  (5–10% prepared from 30%) dropwise to the hot soln. When colorless add 2%  $KMnO_4$  soln slowly until a faint pink or brown persists ca 1 min. If a large amount of  $MnO_2$  forms at this point, again use the peroxide sparingly, then permanganate to discharge the peroxide. Discharge the color from the last permanganate, including a weak brown color from  $MnO_2$ , by adding dropwise just sufficient 8%  $FeSO_4 \cdot 7H_2O$ . Cool to ca  $20^\circ$ , add 3 ml of 0.5 N  $FeNH_4(SO_4)_2 \cdot 12H_2O$  and titrate with the standard thiocyanate. 1 ml of 0.1 N thiocyanate = 0.01003 g of  $Hg$ .

Test for complete extraction of the  $Hg$  from the fat and its removal from the filter, etc., by repeating the  $HNO_3$  (1+1) digestion ca 30 min. on residual fat in flask or on filter, completing this as a separate determination, including the permanganate digestion. Add any titration in excess of 1 to 2 drops (ca 0.05–0.08 ml of 0.1 N  $NH_4CNS$  resulting from this test portion to that obtained by titrating the main extract.

198

MERCURY IN MERCURIAL OINTMENT<sup>102</sup>—TENTATIVE

After mixing the ointment thoroly with a glass rod, avoiding contact with metals, weigh 1 g of the material into an Erlenmeyer flask. Add 20 ml of  $H_2O$  and 20 ml of  $HNO_3$  and heat gently over small flame until red fumes cease to evolve. Cool, and decant the aqueous soln from the ointment base into a separator. Wash ointment base with 50 ml of boiling  $H_2O$ , cool, and decant into separator. Repeat washing until all the Hg is removed. Shake the combined solns in the separator with 50 ml of ether. Transfer the aqueous soln to an Erlenmeyer flask. Wash the ether three times with 10 ml portions of  $H_2O$  until the Hg is removed, adding washings to flask. Add 3 ml of  $FeNH_4(SO_4)_2$ , 186(b), and titrate with 0.1 N  $NH_4CNS$ . 1 ml of 0.1 N  $NH_4CNS$  = 0.01003 g of Hg.

NITRITES IN TABLETS<sup>103</sup>—TENTATIVE

199

## PREPARATION OF SAMPLE

Count and weigh a suitable number of tablets to ascertain the average weight. Reduce to fine powder, mix thoroly, and keep in tightly stoppered bottle.

200

## DETERMINATION

Transfer to a 100 ml volumetric flask a quantity of the powdered sample equivalent to ca 1 g of  $NaNO_2$ , add  $H_2O$  to mark, and mix thoroly. Filter thru a dry filter, rejecting first 10 ml. Transfer a 50 ml aliquot to a 200 ml volumetric flask. Add in order 10 ml of saturated  $KClO_3$  soln, and slowly, with shaking, 10 ml of  $HNO_3$  soln (1+1), and allow to stand 30 min. Add 50 ml of 0.1 N  $AgNO_3$  soln and make up to mark with  $H_2O$ . After mixing thoroly, filter thru a dry filter, rejecting the first 20 ml of filtrate. To 100 ml of filtrate add 2 ml of  $FeNH_4(SO_4)_2$ , 186(b), and titrate with 0.05 N  $NH_4CNS$  soln, 186(a). Make a blank determination, and correct if necessary. 1 ml of 0.1 N  $AgNO_3$  soln = 0.0207 g of  $NaNO_2$ .

NOTE: Correct for any chloride that may be present in sample. If a large quantity of insoluble excipient is present, pipet 100 ml of  $H_2O$  into a flask with the powdered sample in order to avoid any error in volume.

201

PHENOLSULFONATES<sup>104</sup>—OFFICIAL

Dissolve sample (equivalent to ca 0.8 g of phenolsulfonate) in ca 30 ml of  $H_2O$  and add 5 ml of  $HCl$ . Titrate with 0.4 N Br (11.134 g of  $KBrO_3$  + 50 g of  $KBr$  diluted to 1 liter with  $H_2O$ , standardized against 0.1 N  $Na_2S_2O_3$ ). The bromine will be absorbed very rapidly at first, but as the titration proceeds the absorption becomes slower and slower. Titrate as far as possible with no other indicator than the fading of the bromine yellow. (Usually this will be within 1–4 ml of the end point.) Then use methyl orange (0.1%), dropwise, adding no new indicator until the previous drop has practically faded. After adding the reagent, wait a sufficient time for the absorption of the Br before adding more methyl orange (10 seconds at first, 15 seconds at end of titration), because in the presence of dibromophenolsulfonic acid the action of Br on methyl orange is much slower than normal. The end point is reached when, after waiting 15 seconds for the absorption of the last drop of Br and adding a drop of methyl orange, the latter fades very appreciably in 10 seconds. It is always best, after the methyl orange has faded, to add another drop to be sure that the first drop was not added too soon. 1 ml of 0.4 N Br = 0.02321 g of Na phenolsulfonate.

EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE<sup>105</sup>—TENTATIVE

202

## PREPARATION OF SAMPLE

Powder sample, transfer immediately to dry bottle, and seal tightly. Thoroly mix powder in bottle by rotating and shaking before removal of sample for analysis.

Weigh out all needed portions as nearly at the same time as possible. Avoid extreme temperatures and humidities when opening and storing samples.

203

## DETERMINATIONS

(a) *Potassium bromide*.—Weigh 2.5–3 g of the sample and transfer to a 250 ml Erlenmeyer flask. Add 50 ml of  $H_2O$  and swirl gently, avoiding loss of soln by spattering. Acidify the soln with  $HNO_3$  and then add 5 ml in excess. Add 30 ml of 0.1  $N$   $AgNO_3$  soln, VI, 89, and 2 ml of indicator, 186(b). Allow mixture to stand several minutes and swirl occasionally as an aid in flocculating the  $AgBr$ . Titrate excess of 0.1  $N$   $AgNO_3$  with the  $NH_4CNS$  soln, 186(a). 1 ml of 0.1  $N$   $AgNO_3$  = 0.0119 g of  $KBr$ .

(b) *Caffeine*.—Weigh 12–15 g of sample, transfer to a separator, and slowly add 50 ml of  $H_2O$ , avoiding loss of soln by spattering. If soln is not alkaline to litmus, make basic with 5%  $NaOH$  soln. Add 50 ml of  $CHCl_3$ , shake vigorously, and filter into a beaker. Repeat extraction twice, using 50 ml portions of the  $CHCl_3$  each time. Wash filter and funnel with a few ml of  $CHCl_3$  to remove any adhering caffeine. Evaporate combined  $CHCl_3$  filtrate on a water bath to ca 10 ml, finally transferring residual liquid to small weighed beaker. Allow soln to evaporate by gentle heat and an air blast. Dry residue to constant weight at  $80^\circ$ , and weigh.

SILVER PROTEINATES<sup>105</sup>

204

*Acidity or Alkalinity—Tentative*

Dialyze 1 g of sample as directed under 206 and titrate a portion of the clear soln representing 0.5 g of the sample with either 0.02  $N$   $HCl$  or 0.02  $N$   $NaOH$  soln, as required, using phenolphthalein indicator. Calculate acidity as percentage of  $HCl$  and alkalinity as percentage of  $NaOH$ .

205

TOTAL SILVER<sup>107</sup>—OFFICIAL

Place 1 g of sample, accurately weighed, in a 500 ml Kjeldahl flask; add 15 ml of  $H_2SO_4$  and then 10 ml of  $HNO_3$ ; place on steam bath for a few minutes, with occasional rotation, to insure a homogeneous mixture; and boil to white fumes. Add more  $HNO_3$ , boil again to a clear colorless soln, and cool. Add 100 ml of  $H_2O$  and boil until free of nitrogen oxides. Cool, dilute to 300 ml, add 5 ml of  $HNO_3$  and 5 ml of  $FeNH_4(SO_4)_2$  soln, 186(b), and titrate with 0.1  $N$   $NH_4CNS$ . 1 ml of 0.1  $N$   $NH_4CNS$  = 0.01079 g of  $Ag$ .

206 DETECTION AND ESTIMATION OF IONIZABLE SILVER COMPOUNDS<sup>108</sup>—OFFICIAL

Weigh a strip of commercial dialyzing tubing 55 mm wide and ca 1 foot long, wet with  $H_2O$  until uniformly pliable, shake free of adhering  $H_2O$ , and partially dry by rolling in clean paper towel. Reweigh while still moist and place in a 250 ml beaker. (Sheets of dialyzing parchment paper may be used in place of tubing. Over one end of a glass tube 10 cm long and ca 2.5 cm in diameter, fold and secure by means of rubber band a square piece of parchment paper in the form of a sack of sufficient size to hold the sample soln. Dialyzing material should be kept in a humid container to prevent breaking when handled.) Weigh 1 g of the sample, dissolve in 15 ml of  $H_2O$ , and transfer to dialyzing tube. Calculate, and add sufficient  $H_2O$  to beaker to make 100 ml. (This insures 20 ml in the dialyzing tube and 80 ml in the beaker.) Adjust tubing to form a "U" in the beaker, cover with watch-glass, and place in cool dark closet for 24 hours.

(a) *Qualitative Test*.—Test a few ml of the clear, colorless soln from the beaker for  $Ag$  ions by the addition of a few drops of 10%  $HCl$  and a trace of  $HNO_3$ .

(b) *Quantitative Method.*—If Ag ions are present, remove 50 ml of the clear, colorless soln from beaker (representing 0.5 g of sample), dilute to 100 ml, and add 2 ml of  $\text{FeNH}_4(\text{SO}_4)_2$ , 186(b), and the same quantity of colorless  $\text{HNO}_3$ . Titrate with 0.01 N  $\text{NH}_4\text{CNS}$  soln and calculate to percentage by weight of ionizable Ag. 1 ml of 0.01 N  $\text{NH}_4\text{CNS}$  = 0.001079 g of Ag.

207

COD LIVER OIL IN EMULSIONS<sup>109</sup>—TENTATIVE

Weigh into a tared beaker of ca 150 ml capacity sufficient well-mixed sample to contain ca 2 g of cod liver oil. Add ca 10 g of finely powdered  $\text{CaCO}_3$  and thoroly mix with stirring rod. Add 30 ml of  $\text{CHCl}_3$ , throlly mix, and decant thru a dry filter into a 100 ml air-dried, tared beaker. Continue to extract and wash repeatedly with 5–10 ml portions of  $\text{CHCl}_3$  until filtrate is ca 60 ml. Evaporate the  $\text{CHCl}_3$  on steam bath with current of air to ca 5 ml.

Continue extraction and carefully wash filter paper and funnel, filtering into a 250 ml beaker until filtrate is ca 150 ml. Evaporate to ca 10 ml and transfer to first tared beaker. Repeat procedure until extraction is complete or until 25 ml of the solvent upon evaporation in a second tared beaker yields 0.001 g or less of residue. Evaporate the  $\text{CHCl}_3$  in the first tared beaker and allow to remain on steam bath ca 10 min. after the odor of  $\text{CHCl}_3$  has disappeared. Dry in oven at not over 100° for 5 min. intervals until weight is constant or loss is 0.001 g or less.

CAUTION: Avoid prolonged heating or long exposure to air at room temp. The oil absorbs oxygen, the weight increases appreciably, and the physical constants change.

OIL OF CHENOPODIUM<sup>110</sup>—TENTATIVE

208

## REAGENTS

(a) *Standard ferric ammonium sulfate soln.*—Dissolve 39.214 g of pure, crystallized  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  in 200 ml of  $\text{H}_2\text{O}$  in a liter flask, add 30 ml of  $\text{H}_2\text{SO}_4$ , and mix well. Weigh exactly 3.16 g of  $\text{KMnO}_4$ , dissolve the salt in 200 ml of warm  $\text{H}_2\text{O}$ , and slowly add to the soln in the flask, with stirring. (The permanganate soln should be just sufficient to oxidize the iron salt, but it is well to add the last few ml in small portions.) Cool the soln and dilute it to 1 liter with  $\text{H}_2\text{O}$ .

(b) *Standard titanium trichloride soln.*—Add 100 ml of commercial 15–20%  $\text{TiCl}_3$  soln to 200 ml of  $\text{HCl}$ , boil 1 min., cool, and dilute to 4500 ml with  $\text{H}_2\text{O}$ . Place soln in a container with H atmosphere provision and allow to stand 2 days for absorption of residual O. Preserve the  $\text{TiCl}_3$  soln in an atmosphere of H (XXI, Fig. 25), taking care to have all the joints air-tight, and covering the stoppers (preferably counter-sunk) with suitable wax. Standardize by titrating 20 ml of the  $\text{FeNH}_4(\text{SO}_4)_2$  against the  $\text{TiCl}_3$  soln in a protective stream of  $\text{CO}_2$ , using 1 ml of 5%  $\text{NH}_4\text{CNS}$  soln as an indicator. 1 ml of 0.1 N  $\text{FeNH}_4(\text{SO}_4)_2$  = 0.01545 g of  $\text{TiCl}_3$ .

209

## DETERMINATION

Weigh 1 ml of the oil in a 100 ml volumetric flask and dilute to volume with alcohol. Place 50 ml of the  $\text{TiCl}_3$  soln in an Erlenmeyer flask thru which a current of  $\text{CO}_2$  is passing. Fit flask with a Bunsen valve, add 10 ml of the diluted soln of the oil, close flask (with the Bunsen valve), and heat contents almost to boiling 2 min. (Prolonged heating has no effect if the contents are not boiled vigorously.) If the pale violet color of the  $\text{TiCl}_3$  disappears, add more reagent to insure excess. (The formation of a white precipitate does not interfere with the determination.) Add 1 ml of a 5% soln of  $\text{NH}_4\text{CNS}$  and titrate back the excess of  $\text{TiCl}_3$  with the  $\text{FeNH}_4(\text{SO}_4)_2$  in a  $\text{CO}_2$  atmosphere until a faint, permanent, brownish red color is obtained.

Subtract the quantity of  $\text{FeNH}_4(\text{SO}_4)_2$  used, expressed in equivalent mg of

TiCl<sub>3</sub>, from the number of mg of TiCl<sub>3</sub> taken. The difference is the number of mg of TiCl<sub>3</sub> oxidized by oil taken. Convert the mg of TiCl<sub>3</sub> oxidized into ascaridole by dividing by the factor 1.284 (1 g of ascaridole is reduced by 1.284 g of TiCl<sub>3</sub>).

EXAMPLE: 0.9600 g of oil was made up to 100 ml and a 10 ml aliquot was heated with 50 ml of the TiCl<sub>3</sub> soln (1 ml containing 0.0034 g of the salt). It then required 5.9 ml of the reagent, each ml equivalent to 0.01545 g of TiCl<sub>3</sub>, to titrate back the soln. The grams of TiCl<sub>3</sub> oxidized is numerically equal to  $(50 \times 0.0034) - (5.9 \times 0.01545)$ , or 0.07885. The weight of oil in the aliquot was 0.0960 g. Hence the percentage of ascaridole =  $\frac{0.07885 \times 100}{0.096 \times 1.284} = 72.1\%$ .

## BIOASSAY OF DRUGS

### MYDRIATICS AND MYOTICS

#### *Cat-Eye Method*<sup>111</sup>—Official

210

#### APPARATUS

(a) *Mohr pipets*.—1 ml, graduated in 0.1 ml, with slender tips that deliver exactly 0.05 ml per drop.

(b) *N-filled electric lamps*.—100-watt or equally intense illumination.

211

#### ANIMALS

*Adult cats*.—In good physical condition, weighing over 1500 g, and accustomed to being handled.

212

#### PREPARATION OF SAMPLE

Dissolve, in approximately neutral H<sub>2</sub>O, a representative number of tablets, or a sufficient quantity of powder, to make a soln containing 1 mg of the alkaloid per ml soln. If the alkaloids themselves are taken, add the equivalent quantities of acid to convert them into the corresponding salts. Add 2 drops of approximately 0.02 *N* acid per 50 ml of soln.

For great accuracy, the results of chemical assay upon the sample should be followed in the preparation of solns; when such accuracy is unnecessary, the declaration of concentration on the label may be accepted as the basis for the preparation of the soln.

One drop of the respective concentrations of the following drugs is the minimum effective dose:

#### MYDRIATICS

	<i>mg per liter</i>
Atropine.....	12
Hyoscyamine.....	4
Scopolamine.....	0.4
Homatropine.....	200
Cocaine.....	60
Euphthalmin.....	50,000
Ephedrine (alkaloid).....	2,500
Ephedrine salt (or synthetic ephedrine).....	50,000
Pseudoephedrine (alkaloid).....	2,500
Pseudoephedrine (salt).....	80,000

#### MYOTICS

Pilocarpine.....	25,000
Physostigmine (eserine).....	10
Arecoline.....	10,000

213

## DETERMINATION OF CAT'S THRESHOLD

Place a cat ca 1 foot from a 100-watt electric lamp, and determine maximum contractility of its pupils under this condition. Drop 0.05 ml of the freshly prepared standard mydriatic soln, obtained by diluting the 1 mg-per-ml soln, into the outer margin of one eye, leaving the other eye untreated as a control. Compress the inner canthus, while opening and closing the lids, until the fluid has apparently disappeared (10–30 seconds). Return cat to cage.

One and two hours after application (for atropine, 3 and 4 hours also), place cat under the same conditions, and note any differences in diameter between the pupils of the treated and the untreated eyes. (A satisfactory reaction is produced when the pupil of the treated eye is just perceptibly wider (0.5–1.0 mm) than the pupil of the untreated eye.) Do not use the same eye for another assay for at least 24 hours.

If the concentrations given fail to produce a satisfactory reaction, repeat the test with a stronger or weaker soln until the minimum effective concentration is found. (This concentration may vary somewhat for different cats, but it is essentially constant for the same cat.)

214

## BIOASSAY OF UNKNOWN SOLUTIONS

Dilute the 1 mg-per-ml soln to be tested to the minimum effective concentration for the cats to be used, and drop 0.05 ml of this dilution into one eye of the cat, following the same procedure as in the determination of the minimum effective concentration. Also prepare stronger and weaker solns and apply to one eye of each of the other cats used. Test various concentrations until one is obtained that produces satisfactory mydriasis of the same degree as the standard soln when tested on two or more cats.

To obtain the mg of alkaloid present in each ml of the original soln, multiply the mg per ml found to be the cat's minimum effective concentration by the dilution used. Knowing that the original soln was made to contain 0.001 g of alkaloid per ml, calculate the quantity of mydriatic present, and express as percentage of the total alkaloid.

ASSAY OF ERGOT<sup>112</sup>—TENTATIVE

(Applicable to alkaloids of ergotoxine-ergotamine group.)

215

## REAGENTS

- (a) *Menstruum I.*—Mix 20 ml of HCl with 490 ml of alcohol and 490 ml of H<sub>2</sub>O.
- (b) *Menstruum II.*—Diluted alcohol U.S.P.
- (c) *Locke-Ringer soln modified.*—Omit the use of MgCl<sub>2</sub> in the Locke-Ringer soln, U.S.P. XI.

216

## APPARATUS

Use an isolated organ bath similar to that described in the U.S.P. XI under *Liquor Pituitarii Posterii*, modifying it by having two glass chambers for the isolated tissues instead of one. Fix two levers, one above each chamber, to write on the recording drum. The magnification of the two levers should be approximately equal.

217

## PREPARATION OF SAMPLE

Pack the ergot, recently ground to No. 20 powder, in a cylindrical percolator, and slowly percolate with purified petroleum benzin until a few drops of the percolate leave no greasy stain when evaporated from filter paper. Reject the soln, remove drug from percolator, and dry it by exposure to air.

Moisten defatted drug with a 5% soln of  $\text{NaHCO}_3$  and allow to stand in cold room 2-4 hours. Pack in a percolator, add more bicarbonate soln, and allow to macerate 16-24 hours in cold room. Percolate slowly with  $\text{H}_2\text{O}$  until percolate is found by physiological tests to be practically free from amines. Allow to drain completely, remove all but lowest inch of marc from percolator, and remove  $\text{H}_2\text{O}$  from it by strong expression or by centrifuging. Moisten drug with a small quantity of Menstruum I. Repack drug in percolator and macerate overnight in cold room. Add the remainder of Menstruum I, and when this has just disappeared from surface gradually add Menstruum II, constantly maintaining a stratum of liquid above drug. When the liquid begins to drop from percolator, close lower orifice, and having closely covered percolator, macerate 48 hours in a cold room, and then allow percolation to proceed slowly, gradually adding Menstruum II until drug is exhausted. Reserve first 850 ml of percolate, recover alcohol from remainder of percolate, and concentrate the residue to a soft extract at a temp. not exceeding  $60^\circ$  (preferably in a vacuum distillation apparatus). Dissolve extract in reserved portion, mix thoroly, and assay a portion by method given below. From results thus obtained adjust volume of finished fluidextract by addition of Menstruum II to make it conform to required biological standard.

## 218

## THE TEST

Use nonpregnant female rabbits weighing 2 kg or more, and which are at least 3 weeks past parturition. Kill animal by blow on head, cut throat, and suspend by hind legs until hemorrhage ceases. Remove uterus. Cut a piece ca 1 cm in length from one uterine horn. (The remainder of uterine horns may be placed between pieces of cotton wool dampened with the modified Locke-Ringer soln and kept, properly covered, at a temp. of  $40-50^\circ\text{F}$  for any subsequent tests within next 2 days.) Place the piece of uterine horn on convex surface of watch-glass and cut it open along the line of mesometric attachment. Unfurl, and cut away the sides so that a piece 8-10 mm wide farthest from the mesometrium remains. Divide this piece longitudinally into two equal parts and suspend each in one of the glass containers of the bath holding the Locke-Ringer soln. Weight recording levers so as to induce relaxation of the muscle. To each bath add 0.02 mg of epinephrine (as the hydrochloride in soln). If necessary, increase simultaneously the dose in each bath by increments of 0.01 or 0.02 mg until a contraction which is maintained 2-3 min. is produced. Adjust weights on levers so that extent of contraction in each bath is similar and conspicuously greater than any spontaneous contractions. Spontaneous contractions increase in amplitude as muscle remains in bath so that weighting should as a rule be greater than at first seems necessary. At a noted time add to one bath a dose of a specific alkaloid. (This may be 0.4 ml of a soln of ergotamine tartrate or ergotoxine phosphate, 1 in 30,000. The concentration may vary from 1 part in 10,000 to 1 part in 200,000, depending upon the response obtained in the muscle.) 30 seconds later add to the other bath 0.4 ml of the unknown soln diluted approximately 30 times; 10 min. from the time of the additions to each bath add epinephrine to each bath in the dosage found to give a previously satisfactory contraction. Note the contraction produced on each muscle. If the contraction has been reduced to the same extent in both baths the concentration of the specific alkaloid in each bath is the same. If the contractions are not the same, repeat the procedure, using fresh uterine strips, the same dose of epinephrine and larger or smaller doses of the ergot preparation as indicated by the previous test.

MICROCHEMICAL TESTS FOR ALKALOIDS<sup>112</sup>—OFFICIAL OR TENTATIVE

219

## REAGENTS

- (a) *Sodium carbonate soln.*—Dissolve 5 g of  $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (b) *Potassium iodide soln.*—Dissolve 5 g of KI in 100 ml of  $\text{H}_2\text{O}$ .
- (c) *Gold chloride soln.*—Dissolve 1 g of gold chloride in 20 ml of  $\text{H}_2\text{O}$ .
- (d) *Hydrochloric acid.*—5%.
- (e) *Kraut's*—Dissolve 8 g of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  in 20 ml of  $\text{HNO}_3$ , sp. gr. 1.18. Dissolve 27.2 g of KI in 50 ml of  $\text{H}_2\text{O}$ . Mix the solns and dilute to 100 ml.
- (f) *Wagner's (iodine).*—Dissolve 1.27 g of I and 2 g of KI in 5 ml of  $\text{H}_2\text{O}$  and dilute to 100 ml.
- (g) *Potassium ferrocyanide soln.*—Dissolve 5 g of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (h) *Ammonium thiocyanate soln.*—Dissolve 5 g of  $\text{NH}_4\text{CNS}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (i) *Mercuric chloride soln.*—Dissolve 5 g of  $\text{HgCl}_2$  in 100 ml of  $\text{H}_2\text{O}$ .
- (j) *Sodium benzoate soln.*—Dissolve 5 g of Na benzoate in 100 ml of  $\text{H}_2\text{O}$ .
- (k) *Platinic chloride soln.*—Dissolve 5 g of  $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (l) *Disodium phosphate soln.*—Dissolve 5 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (m) *Marmé's.*—Dissolve 3 g of  $\text{CdI}_2$  in 18 ml of  $\text{H}_2\text{O}$  containing 6 g of KI.
- (n) *Potassium permanganate soln.*—Dissolve 1 g of  $\text{KMnO}_4$  in 100 ml of  $\text{H}_2\text{O}$ .
- (o) *Zinc potassium iodide soln.*—Dissolve 5 g of  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$  and 20 g of KI in 100 ml of  $\text{H}_2\text{O}$ .
- (p) *Potassium hydroxide soln.*—Dissolve 5 g of KOH in 100 ml of  $\text{H}_2\text{O}$ .
- (q) *Ammonium hydroxide soln.*—10%.
- (r) *Mercuric chloride-sodium chloride soln.*—Dissolve 5 g of  $\text{HgCl}_2$  and 0.75 g of NaCl in 100 ml of  $\text{H}_2\text{O}$ .
- (s) *Zinc chloride soln.*—Dissolve 5 g of  $\text{ZnCl}_2$  in 100 ml of  $\text{H}_2\text{O}$ .
- (t) *Ammoniacal silver nitrate soln.*—Dissolve 2 g of  $\text{AgNO}_3$  in 100 ml of 5%  $\text{NH}_4\text{OH}$ . Prepare fresh soln each time used.
- (u) *Lead iodide soln.*—To a 1:3 K acetate soln in  $\text{H}_2\text{O}$ , add a drop of methyl red indicator and acetic acid until the yellow color changes to orange; then, while gently warming, saturate with  $\text{PbI}_2$ , cool, and filter.

220

## PREPARATION OF SAMPLES

- (a) *Controls.*—Dissolve 1 mg of the pure alkaloidal salt in 2 drops of  $\text{H}_2\text{O}$  to make an approximately 1–100 soln.
- (b) *Alkaloids in compounds.*—Separate the alkaloid in pure form by extracting it from ammoniacal soln with a suitable immiscible solvent, and evaporate the solvent. To 1 mg of the residue add, dropwise, 0.1 N HCl, avoiding an excess of acid, and dilute with  $\text{H}_2\text{O}$ , if necessary, to approximately the same alkaloidal concentration as is specified in (a).
- (c) *Hypodermic tablets.*—Dissolve a portion of a tablet in  $\text{H}_2\text{O}$  and dilute with  $\text{H}_2\text{O}$  to approximately the same alkaloidal concentration as specified in (a).

221

## IDENTIFICATION

Place a drop of the alkaloidal soln on a clean glass slide, add a drop of reagent by means of a clean glass rod, and without stirring or covering examine under microscope, using low power. A magnification of 100–150 is suitable. Note kind of crystals formed and compare their characteristics with descriptions given and also with a control (see table under 222).



*Characteristics of Microchemical Tests for Alkaloids*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Aconitine <sup>113</sup>	Sodium carbonate	In 1:3000 soln heated to 50° in test tube. Small transparent hexagonal plates, also rods in contact.
Apomorphine <sup>114</sup>	Potassium iodide Gold chloride Hydrochloric acid	1:50. Small crystals, which have sharp clear cut angles like those of a diamond. Red-brown, fine needles, in dense masses in all solns to 1:10,000. 1:50. Small rods singly and in clusters.
Arecoline <sup>115</sup>	Kraut's	Red, rhombic crystals.
Atropine <sup>116</sup>	Wagner's	Small dark rods and triangular plates form in great numbers, singly and in groups.
Benzocaine <sup>117</sup>	Potassium ferrocyanide	1:100 in dilute HCl. Colorless, irregular plates and rods.
Benzyl morphine <sup>118</sup> (Peronine)	Potassium iodide  Ammonium thiocyanate Hydrochloric acid	1:200. Dense rosettes of needles. Crystals are formed readily in dilute solns (1:1000) in form of sheaves of needles. 1:200. Rosettes and sheaves of needles in acid or neutral soln. 1:100. Rods, usually notched at ends and often in rosettes, are formed on stirring.
Berberine <sup>*119</sup>	Hydrochloric acid	Saturated soln; fine yellow needles. (Avoid excess reagent.)
Brucine <sup>120</sup>	Potassium iodide Mercuric chloride	Long masses of transparent, rectangular plates; also rosettes of thin plates. Small, dense rosettes.
Caffeine <sup>120</sup>	Mercuric chloride	Clusters of long, radiating, needle-shaped crystals.
Cinchonidine <sup>121</sup>	Sodium benzoate  Platinic chloride Sodium carbonate	Rosettes and sheaves of needles spreading to large size. Rosettes of transparent plates. Spherical crystals, but not needles as in cinchonine.
Cinchonine <sup>121</sup>	Sodium carbonate  Disodium phosphate	Dark rosettes, composed of radiating needles, form immediately. Similar to crystals formed by sodium carbonate, but more burr-shaped.
Cocaine <sup>122</sup>	Platinic chloride	Delicate, feathery crystals; later becoming heavier in structure.
Codeine <sup>122</sup>	Marmé's  Wagner's	Silvery, circular masses, crystallizing into dark rosettes of irregular outline. Heavy, red-brown precipitate; crystallizes very slowly in yellow blades extending in branches (never red).

\* Tentative, all others official methods.

*Characteristics of Microchemical Tests for Alkaloids—Continued*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Cotarnine* <sup>123</sup>	Platinic chloride Mercuric chloride Potassium ferrocyanide	1:200. Hair-like crystals, yellow and curving. Colorless, long, branching needles. Acidified with 1 drop of 5% HCl; globules that develop into dense, burr-shape crystals; also amber-brown plates.
Ephedrine <sup>124</sup>	Kraut's	Long, brown radiating and interlacing needles.
Ethylhydrocupreine <sup>125</sup>	Ammonium thiocyanate	1:100 in 0.1 N HCl. Long, straight needles.
Ethylmorphine <sup>126</sup> (Dionine)	Wagner's Mercuric chloride	1:200. Groups of yellow needles, branching later. Transparent plates often with notched ends; singly and in groups. Stir to start crystallization.
Heroine <sup>127</sup>	Platinic chloride	Spherical clusters of golden yellow needles form slowly around a nucleus; cluster disintegrates on standing.
Homatropine <sup>128</sup>	Gold chloride	1:200. Green-gold blades, often with pointed ends and united in pairs; surfaces appear etched on long standing.
Hydrastine <sup>129</sup>	One drop of 5% HCl and potassium ferrocyanide	1:100. Spheres of radiating crystals. Shake slide to start crystallization. Avoid excess reagent.
Hydrastinine <sup>130</sup>	Potassium permanganate  Mercuric chloride  One drop of 5% hydrochloric acid and potassium ferrocyanide	1:500. Immediate red plates, often with serrated edges. In concentrated soln, great number of large red or brown plates with deeply cut edges. 1:500. Transparent needles forming branches rapidly in neutral and acidified solns. 1:200. Yellow rhombic plates and tree-like crystals.
Hyoscyamine <sup>131</sup>	Gold chloride	Thin, transparent, nearly colorless irregular plates, often curved. Crystals form slowly in 1:100 to 1:200 soln. Shaking the slide aids crystallization.
Morphine <sup>132</sup>	Marmé's Wagner's	Silvery, gelatinous precipitate, crystallizing in dense masses of fine needles. Small drop of reagent produces heavy, red-brown precipitate, slowly crystallizing in shining, red, overlapping plates extending in branches.
Narceine* <sup>133</sup>	Wagner's, or zinc potassium iodide Platinic chloride	1:400. Blue, radiating needles, sometimes with yellow dichroism. Beautiful, feathery rosettes develop in all solns.

*Characteristics of Microchemical Tests for Alkaloids—Continued*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Narcotine <sup>*133</sup>	Potassium hydroxide or ammonium hydroxide	1:200. White amorphous precipitate, which crystallizes slowly; dense rosettes of needles.
Nicotine <sup>134</sup>	Mercuric chloride  Mercuric chloride-sodium chloride	Radiating transparent blades form in presence of slight excess of H <sub>2</sub> SO <sub>4</sub> ; feather-like blades form in presence of HCl. Radiating transparent blades.
Papaverine <sup>135</sup>	Zinc chloride	Thin rectangular plates in excess HCl
Physostigmine <sup>*135</sup>	Lead iodide	1:100 soln. Radiating serrated plates.
Pilocarpine <sup>137</sup>	Platinic chloride	Crystals form slowly; layers of thin, yellow, triangular plates of delicate structure.
Procaine hydrochloride <sup>138</sup>	Platinic chloride  Gold chloride and hydrochloric acid	Spherical crystals of radiating branches  Irregular, radiating branches.
Quinidine <sup>139</sup>	Potassium iodide	Small, triangular crystals in great numbers; best in 1:1000 dilution; soluble in excess reagent.
Quinine <sup>139</sup>	Disodium phosphate	Silvery, sheaf-like crystals.
Scopolamine <sup>140</sup> (Hyoscyne)	Gold chloride	Clusters of pale yellow, transparent blades, with coarse, saw-toothed edges form immediately on shaking the slide. Crystals grow to large size in 1:200 soln.
Sparteine <sup>141</sup>	Gold chloride	Large numbers of blade-like crystals varying in size according to concentration.
Stovaine <sup>*142</sup>	To soln add drop of HCl and gold chloride	1:50. Tree-like crystals.
Strychnine <sup>143</sup>	Platinic chloride  Marmé's	Crystals form immediately in clusters and singly in small, wedge-shaped needles, which move about the field. Silvery masses, slowly forming rosettes.
Theobromine <sup>144</sup>	Kraut's (freshly prepared)	In hydrochloric acid (1+3). Tufts of brown radiating needles form readily in 1:200 soln.
Theophylline <sup>145</sup>	Ammoniacal silver nitrate Mercuric chloride	1:200. Gelatinous at first; dense spheres of dark radiating needles. 1:150. Spheres and double tufts of dense radiating needles.
Yohimbine <sup>146</sup>	Sodium carbonate	In 1:1000 soln heated to 50°. Fine needles in sheaf-like bundles and rosettes.

## REAGENTS

- (a) *Phosphotungstic acid soln.*—Dissolve 5 g of  $P_2O_5$ ,  $24WO_3 \cdot xH_2O$  in 100 ml of  $H_2O$ .
- (b) *Bromide-bromate soln.*—Dissolve 0.3 g of  $KBrO_3$  and 1.2 g of  $KBr$  in  $H_2O$  and dilute to 100 ml.
- (c) *Nitric acid.*—Mix one volume of  $HNO_3$  with one volume of  $H_2O$ .
- (d) *Wagner's.*—See 219(f).
- (e) *Silver nitrate soln.*—Dissolve 1 g. of  $AgNO_3$  in 20 ml of  $H_2O$ .
- (f) *Mercuric chloride soln.*—See 219(i).
- (g) *Marmé's.*—See 219(m).
- (h) *Acetic acid.*—Dilute 6 ml of glacial acetic acid to 100 ml with  $H_2O$ .
- (i) *Potassium ferrocyanide soln.*—See 219(g).
- (j) *Ammoniacal silver nitrate soln.*—See 219(t).
- (k) *Gold chloride soln.*—See 219(c).
- (l) *Lead triethanolamine soln.*—Add 1 ml of triethanolamine (tech. 90% is satisfactory) to a soln of 1 g of neutral Pb acetate in 20 ml of  $H_2O$ . A slight turbidity does not interfere.
- (m) *Zinc pyridine soln.*—Add 1 ml of pyridine to a soln of 1 g of Zn acetate in 20 ml of  $H_2O$ .
- (n) *Barium hydroxide soln.*—Saturated soln in  $H_2O$ .
- (o) *Silicotungstic acid soln.*—Dissolve 5 g of  $4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 22H_2O$  in 100 ml of approximately 6 N  $H_2SO_4$ .
- (p) *Lead acetate soln.*—Dissolve 5 g of Pb acetate in  $H_2O$  to make 100 ml.
- (q) *Mercurous nitrate soln.*— $HgNO_2$  test soln.
- (r) *Ammonium thiocyanate soln.*—See 219(h).
- (s) *Platinic chloride soln.*—See 219(k).
- (t) *Magnesia mixture.*—Dissolve 55 g of  $MgCl_2 \cdot 6H_2O$  and 140 g of  $NH_4Cl$  in  $H_2O$ . Add 130.5 ml of  $NH_4OH$  and  $H_2O$  to make 1 liter.
- (u) *Ammoniacal nickel acetate soln.*—Mix 1 volume of 5%  $Ni(C_2H_3O_2)_2$  with 1 volume of 10%  $NH_4OH$ . Use the clear, supernatant liquid.
- (v) *Benzaldehyde.*—N.F. quality.
- (w) *Sodium nitrite soln.*—Dissolve 10 g of  $NaNO_2$  in  $H_2O$  to make 100 ml.
- (x) *Kraut's.*—See 219 (e).

## Characteristics of Microchemical Tests for Synthetics

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Acetanilid <sup>147</sup>	10% HCl	1:100	Phosphotungstic acid	Rosettes of prisms.
	10% HCl	1:100	Bromide-bromate soln	Small prisms.
Acetophenetidin <sup>147</sup>		About 1 mg of the powdered material	Nitric acid	After adding a drop of nitric acid let stand for a few seconds, then add a drop of $H_2O$ . Bright yellow, curving, branched crystals.
	10% HCl	Saturated soln	Wagner's	Large, irregular plates.
Acetylsalicylic acid <sup>148</sup>	2% triethanolamine	1:50	Silver nitrate	Fine, curling, hair-like crystals form first near edge of the drop.
Aminopyrine <sup>149</sup>	$H_2O$	1:100	Mercuric chloride Marmé's	Long, slender radiating crystals, often curved. Groups of spiny branches.

*Characteristics of Microchemical Tests for Synthetics—Continued*

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Amytal <sup>150</sup>	3% NH <sub>4</sub> OH	1:50	Acetic acid	Long branching needles; some hexagonal plates in groups. Groups of rectangular plates.
	3% NH <sub>4</sub> OH	1:25	Acetic acid	
Antipyrine <sup>151</sup>	H <sub>2</sub> O	1:100	Potassium ferrocyanide	Acicular and prismatic crystals form after a drop of 1% HCl is added.
Barbital <sup>151</sup>	About 1 mg of powder	—	Ammoniacal silver nitrate	Stir to aid solution and crystallization. Very small twinned crystals and larger tufts. Dark burrs (stirring hastens crystallization).
	3% NH <sub>4</sub> OH	1:50	Acetic acid	
Benzoic acid <sup>152</sup>	Dry powder	—	Lead triethanolamine	Stir a small quantity of the synthetic into a drop of reagent. Stir thoroly to induce crystallization. 4-sided plates, singly and in groups. Stir a small quantity of synthetic into a drop of reagent. Stir thoroly to induce crystallization. Hexagonal crystals.
	Dry powder	—	Zinc pyridine	
Cinchophen <sup>153</sup>	0.1 N NaOH Add H <sub>2</sub> O, and make slightly acid with HCl 2% triethanolamine	1:1000	Gold chloride	Dark clusters of needles. Few short, rhombic crystals.
		1:100	Silver nitrate	Rods or curving blades with irregular ends.
Dinitrophenol <sup>154</sup>	Small quantity of 0.1 N NaOH	1:100	Hydrochloric acid	Plates with four branches. In more dilute soln single, rectangular plates.
Diallylbarbituric acid <sup>155</sup>	Dry powder	—	Lead triethanolamine	Stir a small quantity of the synthetic into a drop of the reagent. Rods singly and in clusters. Stir a small quantity of the synthetic into a drop of the reagent. Rods singly and in groups.
	Dry powder		Barium hydroxide	
Hydroxyquinoline sulfate <sup>156</sup> (Chinosol)	Dissolve the salt in H <sub>2</sub> O. If free base, dissolve in 10% HCl, avoiding excess	1:500	Magnesia mixture	Small, elliptical grains. Few burr-shaped crystals on standing.

*Characteristics of Microchemical Tests for Synthetics—Continued*

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Mandelic acid <sup>157</sup>	H <sub>2</sub> O soln	1:100	Lead acetate	Rosettes of thin curving plates. Burr-shaped groups of needles.
	H <sub>2</sub> O soln	1:100	Mercurous nitrate	
Methen-amine <sup>158</sup>	H <sub>2</sub> O	1:500	Silicotungstic acid	Thin transparent, rectangular crystals.
Neocinchophen <sup>159</sup>	10% HCl	Saturated soln	Ammonium thiocyanate	Rosettes of needles. (Gentle agitation by tipping the slide back and forth hastens crystallization.) Needles in clusters.
	10% HCl	Saturated soln	Platinic chloride	
Phenobarbital <sup>160</sup>	About 1 mg of powder	—	Ammoniacal nickel acetate	Stir to aid solution and crystallization. Single rectangular crystals.
Pyridium <sup>161</sup>	Dissolve the salt in H <sub>2</sub> O. If free base, dissolve in 10% HCl, avoiding excess	1:1000	Ammonium thiocyanate	Small, red-brown dense sheaves.
Salicylic acid <sup>162</sup>	Dry powder	10% HCl	Bromide-bromate soln	Stir a few crystals of the synthetic into a drop of the HCl. Add a drop of the bromide-bromate soln. Fine needles appear to grow from the crystals of salicylic acid. Stir a few crystals into a drop of the reagent. Rods or needles grow from the crystal of salicylic acid. Small irregular plates; a few short rods.
	Dry powder	—	Lead triethanolamine	
	2% triethanolamine	1:100 to 1:200	Silver nitrate	
Sulfanilamide <sup>163</sup>	Dry powder		Benzaldehyde	Stir thoroly a small amount of synthetic into a drop of reagent. 4-sided plates. Yellow needles.
	0.1 N HCl	Saturated soln	Sodium nitrite	
Triethanolamine <sup>164</sup>	H <sub>2</sub> O	1:100	Kraut's	Oily globules changing to large, red, hexagonal plates and prismatic crystals.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 10, 99 (1927).
- <sup>2</sup> J. Ind. Eng. Chem., 6, 665 (1914).
- <sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. 132, p. 197; J. Assoc. Official Agr. Chem., 17, 78 (1934).
- <sup>4</sup> U. S. Dept. Agr. Bur. Chem. Bull. 162, p. 195.
- <sup>5</sup> Ibid., p. 200.
- <sup>6</sup> Ibid., 152, p. 195.
- <sup>7</sup> Ibid., 162, p. 193.
- <sup>8</sup> J. Ind. Eng. Chem., 7, 681 (1915).
- <sup>9</sup> J. Assoc. Official Agr. Chem., 5, 582 (1922).
- <sup>10</sup> Ibid., 583.
- <sup>11</sup> Ibid., 8, 499 (1925); 9, 49 (1926).
- <sup>12</sup> Ibid., 50.
- <sup>13</sup> Ibid., 22, 91 (1939).
- <sup>14</sup> Ibid., 23, 56 (1940).
- <sup>15</sup> Ibid., 21, 560 (1938); 22, 95, 732 (1939).
- <sup>16</sup> Ibid., 13, 47 (1930).
- <sup>17</sup> Ibid., 7, 29 (1923); 8, 40 (1924).
- <sup>18</sup> Ibid., 11, 51 (1928).
- <sup>19</sup> J. Ind. Eng. Chem., 7, 519 (1915).
- <sup>20</sup> J. Assoc. Official Agr. Chem., 8, 48, 510 (1925); 9, 51 (1926); 19, 106 (1936).
- <sup>21</sup> Ibid., 19, 106 (1936).
- <sup>22</sup> Ibid., 9, 52 (1926).
- <sup>23</sup> J. Ind. Eng. Chem., 11, 756 (1919).
- <sup>24</sup> Ibid., 14, 24 (1922).
- <sup>25</sup> Ibid., 10, 606 (1918).
- <sup>26</sup> J. Assoc. Official Agr. Chem., 7, 20 (1923); 23, 59 (1940).
- <sup>27</sup> Ibid., 9, 318 (1926); 10, 47 (1927).
- <sup>28</sup> Ibid., 15, 81 (1932).
- <sup>29</sup> Ibid., 23, 89 (1940).
- <sup>30</sup> Ibid., 16, 83 (1933).
- <sup>31</sup> Ibid., 15, 402 (1932); 18, 84 (1935).
- <sup>32</sup> Methods of Analysis, A.O.A.C., 1925, p. 390; J. Official Agr. Chem., 13, 65 (1930).
- <sup>33</sup> J. Official Agr. Chem., 8, 44 (1924).
- <sup>34</sup> Ibid., 11, 49 (1928); 23, 90 (1940).
- <sup>35</sup> Ibid., 14, 358 (1931).
- <sup>36</sup> Ibid., 328.
- <sup>37</sup> Ibid., 13, 329 (1930).
- <sup>38</sup> Ibid., 18, 88 (1935).
- <sup>39</sup> Ibid., 21, 95 (1938); Ibid., 22, 100 (1939); 23, 59 (1940).
- <sup>40</sup> Ibid., 10, 49 (1927).
- <sup>41</sup> Ibid., 5, 152 (1921).
- <sup>42</sup> Ibid., 154.
- <sup>43</sup> Ibid., 151.
- <sup>44</sup> Ibid., 17, 446 (1934); 18, 88 (1935); 23, 59 (1940).
- <sup>45</sup> Ibid., 12, 54 (1929).
- <sup>46</sup> Ibid., 5, 163, 589 (1921-22).
- <sup>47</sup> Ibid., 23, 58 (1940).
- <sup>48</sup> Methods of Analysis, A.O.A.C., 1925, p. 407; J. Assoc. Official Agr. Chem., 17, 79 (1934); 19, 106 (1936).
- <sup>49</sup> Methods of Analysis, A.O.A.C., 1925, p. 404.
- <sup>50</sup> J. Assoc. Official Agr. Chem., 9, 53 (1926); 19, 105 (1936).
- <sup>51</sup> Ibid., 19, 105 (1936).
- <sup>52</sup> Ibid., 21, 555 (1938); 22, 94 (1939).
- <sup>53</sup> Ibid., 20, 82 (1937).
- <sup>54</sup> Ibid., 15, 407 (1932).
- <sup>55</sup> Ibid., 16, 81 (1933).
- <sup>56</sup> Ibid., 12, 291 (1929).
- <sup>57</sup> Ibid., 22, 92 (1939).
- <sup>58</sup> Ibid., 11, 50 (1928).
- <sup>59</sup> Ibid., 18, 89 (1935).
- <sup>60</sup> Ibid., 15, 83 (1932).

- <sup>61</sup> Ibid., 12, 300 (1929).  
<sup>62</sup> Ibid., 54.  
<sup>63</sup> Ibid., 15, 81 (1932).  
<sup>64</sup> U. S. Dept. Agr., Bur. Chem. Circ., 94, p. 3.  
<sup>65</sup> J. Assoc. Official Agr. Chem., 19, 535 (1936); 21, 557 (1938); 22, 95, 730 (1939).  
<sup>66</sup> Ibid., 14, 360 (1931); 22, 761 (1939); Am. J. Pharm., 101, 654 (1929).  
<sup>67</sup> J. Assoc. Official Agr. Chem., 12, 50, 264 (1929).  
<sup>68</sup> Ibid., 17, 78 (1934).  
<sup>69</sup> Ibid., 16, 357 (1933).  
<sup>70</sup> Ibid., 21, 95 (1938).  
<sup>71</sup> Ibid., 20, 83 (1937).  
<sup>72</sup> Ibid., 82.  
<sup>73</sup> Ibid., 21, 543 (1938); 22, 100 (1939).  
<sup>74</sup> Ibid., 20, 81 (1937).  
<sup>75</sup> Ibid., 22, 98 (1939).  
<sup>76</sup> Ibid., 23, 58 (1940).  
<sup>77</sup> Ibid., 12, 49 (1929).  
<sup>78</sup> Ibid., 7, 14 (1923); 22, 99 (1939).  
<sup>79</sup> J. Ind. Eng. Chem., 12, 766 (1920); J. Assoc. Official Agr. Chem., 8, 541 (1925).  
<sup>80</sup> J. Assoc. Official Agr. Chem., 21, 94 (1938).  
<sup>81</sup> Ibid., 22, 97 (1939).  
<sup>82</sup> Ibid., 14, 321 (1931).  
<sup>83</sup> Ibid., 18, 87 (1935).  
<sup>84</sup> Ibid., 19, 104 (1936).  
<sup>85</sup> Ibid., 10, 44 (1927).  
<sup>86</sup> Ibid., 11, 48 (1928).  
<sup>87</sup> Ibid., 49.  
<sup>88</sup> Ibid., 15, 84 (1932).  
<sup>89</sup> Ibid., 17, 75 (1934).  
<sup>90</sup> Ibid., 18, 87 (1935).  
<sup>91</sup> Ibid., 22, 90 (1939).  
<sup>92</sup> Ibid., 15, 83 (1932).  
<sup>93</sup> Ibid., 21, 94 (1938).  
<sup>94</sup> Ibid., 14, 377 (1931).  
<sup>95</sup> Ibid., 15, 85 (1932).  
<sup>96</sup> Ibid., 11, 51 (1928); 12, 52 (1929).  
<sup>97</sup> Ibid., 14, 312 (1931).  
<sup>98</sup> Ibid., 12, 52 (1929).  
<sup>99</sup> Ibid., 17, 75 (1934).  
<sup>100</sup> Ibid., 76.  
<sup>101</sup> Ibid., 22, 96 (1939).  
<sup>102</sup> Ibid., 13, 364 (1930); 14, 351 (1931); 15, 342 (1932), 18, 85 (1935).  
<sup>103</sup> Ibid., 18, 89 (1935).  
<sup>104</sup> Ibid., 16, 83, 292, 364 (1933).  
<sup>105</sup> Ibid., 21, 96 (1938).  
<sup>106</sup> Ibid., 11, 53 (1928).  
<sup>107</sup> Ibid., 8, 551 (1925); 9, 54 (1926).  
<sup>108</sup> Ibid., 8, 551 (1925); 9, 55 (1926); 10, 46 (1927).  
<sup>109</sup> Ibid., 22, 96 (1939).  
<sup>110</sup> Ibid., 13, 334 (1930); 14, 53 (1931).  
<sup>111</sup> Ibid., 11, 53 (1928).  
<sup>112</sup> Methods of Analysis, A.O.A.C., 1930, p. 487; J. Assoc. Official Agr. Chem., 14, 51 (1931).  
<sup>113</sup> J. Assoc. Official Agr. Chem., 15, 81 (1932).  
<sup>114</sup> Ibid., 20, 80 (1937); 21, 92 (1938).  
<sup>115</sup> Ibid., 15, 81 (1932).  
<sup>116</sup> Ibid., 11, 354 (1928); 12, 284 (1929); 14, 51, 317 (1931).  
<sup>117</sup> Ibid., 16, 84 (1933).  
<sup>118</sup> Ibid., 21, 91 (1938).  
<sup>119</sup> Ibid., 22, 89 (1939).  
<sup>120</sup> Ibid., 13, 46 (1930).  
<sup>121</sup> Ibid., 12, 53 (1929).  
<sup>122</sup> Ibid., 10, 372 (1927); 11, 52 (1928).  
<sup>123</sup> Ibid., 22, 89 (1939).



- <sup>124</sup> Ibid., 14, 817 (1931).  
<sup>125</sup> Ibid., 20, 80 (1937).  
<sup>126</sup> Ibid., 21, 92 (1938).  
<sup>127</sup> J. Ind. Eng. Chem., 4, 508 (1912); J. Assoc. Official Agr. Chem., 5, 154 (1921);  
10, 372 (1927); 11, 52, 354 (1928).  
<sup>128</sup> J. Assoc. Official Agr. Chem., 18, 85 (1935).  
<sup>129</sup> Ibid., 20, 80 (1937).  
<sup>130</sup> Ibid., 21, 92 (1938).  
<sup>131</sup> Ibid., 18, 86 (1935).  
<sup>132</sup> Ibid., 10, 372 (1927); 11, 52, 354 (1928).  
<sup>133</sup> Ibid., 22, 88 (1939).  
<sup>134</sup> Ibid., 16, 82 (1933).  
<sup>135</sup> Ibid., 17, 76 (1934).  
<sup>136</sup> Ibid., 23, 55 (1940).  
<sup>137</sup> Ibid., 11, 354 (1928).  
<sup>138</sup> Ibid., 17, 76 (1934).  
<sup>139</sup> Ibid., 12, 53 (1929).  
<sup>140</sup> Ibid., 18, 86 (1935).  
<sup>141</sup> Ibid., 16, 82 (1934).  
<sup>142</sup> Ibid., 23, 55 (1940).  
<sup>143</sup> Ibid., 11, 52, 354 (1928).  
<sup>144</sup> Ibid., 19, 103 (1936).  
<sup>145</sup> Ibid., 19, 103 (1936); 20, 80 (1937).  
<sup>146</sup> Ibid., 15, 81 (1932).  
<sup>147</sup> Ibid., 19, 103 (1936).  
<sup>148</sup> Ibid., 21, 93 (1938).  
<sup>149</sup> Ibid., 18, 86 (1935).  
<sup>150</sup> Ibid., 20, 80 (1937).  
<sup>151</sup> Ibid., 17, 77 (1934).  
<sup>152</sup> Ibid., 21, 93 (1938).  
<sup>153</sup> Ibid., 16, 84 (1933).  
<sup>154</sup> Ibid., 18, 86 (1935).  
<sup>155</sup> Ibid., 22, 90 (1939).  
<sup>156</sup> Ibid., 16, 84 (1933).  
<sup>157</sup> Ibid., 22, 90 (1939).  
<sup>158</sup> Ibid., 17, 77 (1934).  
<sup>159</sup> Ibid., 19, 104 (1936).  
<sup>160</sup> Ibid., 20, 81 (1937).  
<sup>161</sup> Ibid., 16, 84 (1933).  
<sup>162</sup> Ibid., 21, 93 (1938).  
<sup>163</sup> Ibid., 22, 90 (1938).  
<sup>164</sup> Ibid., 17, 77 (1934).

## **XL. MICROBIOLOGICAL METHODS**

### **EXAMINATION OF FROZEN EGG PRODUCTS<sup>1</sup>**

The Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists should be used as a guide for the further study of microorganisms obtained in the cultural procedures described.

#### **SAMPLING**

**1**

##### **EQUIPMENT**

(a) Electric or hand drill with auger (1"×16"), (b) alcohol burner, (c) alcohol (95% C<sub>2</sub>H<sub>5</sub>OH), (d) absorbent cotton, (e) two tablespoons, (f) sample containers (sterile 1 pt. or 1 qt. Mason jars), (g) hammer and steel strip (12"×2"×0.25") or other tool for opening egg cans, (h) water pail, (i) towels, (j) record book, pencils, etc.

**2**

##### **PROCEDURE**

Select a representative number of cans from lot (square root of total). Note and record all marks of identification, for example: firm name and location, brand, type product, code, or lot numbers, etc. Sterilize auger and tablespoons by sponging off with alcohol-soaked absorbent cotton and heating in flame of alcohol burner. Wash drill and spoons in pail of water and re-sterilize after sampling each container. Open cans aseptically. Drill three cores from top to bottom of can; one core in the center, a second midway between center and periphery, and a third core near edge of can. Transfer drillings from can to sample container with sterile tablespoon. Examine product organoleptically by smelling at the opening of a fourth drill-hole made after removal of bacteriological sample. (Heat produced by electric drill intensifies odor of egg material, thus facilitating organoleptic examination.) Record odors as normal, putrid, sour, or musty. Refrigerate samples with dry ice if analysis is to be delayed or sampling point is at some distance from laboratory. Carry out sampling procedure under as nearly aseptic conditions as possible.

#### **ANALYTICAL PROCEDURE**

**3**

##### **PREPARATION OF SAMPLE**

Thaw frozen egg material as rapidly as possible in order to prevent increase in numbers of microorganisms present and at temp. sufficiently low to prevent destruction of microorganisms (37°). (Frequent rotary shaking of sample container aids in thawing frozen material. Thawing temp. may be maintained by the use of a water bath or bacteriological incubator.) Thoroughly mix each thawed sample with the sterile spoon or electric stirrer before analysis. Prepare 1-10 dilution by aseptically weighing 11 g of egg material into a wide-mouthed glass-stoppered bottle containing 99 g of sterile physiological salt soln and 1 tablespoonful of glass shot. Agitate the dilution thoroly to insure complete soln or distribution of egg material in diluent by shaking container rapidly 100 times, each shake being an up-and-down excursion of about a foot, and time interval not exceeding 45 seconds. Also use the 1-10 dilution for preparation of serial dilutions from 1-100 to 1-100,000,000 for inoculation into various culture media. Use sterile physiological salt soln in preparing all dilutions. Pour all plates and inoculate other media within 15 min. after preparation

of first dilution in order to prevent growth or death of microorganisms in the dilution bottles or tubes, thus affecting final results.

4

## PLATE COUNTS

Inoculate triplicate plates with 1 ml portions of all dilutions from 1-10 to 1-1,000,000. Pour plates with nutrient agar, previously cooled to 40-45°. Incubate one set of plates at 20°, one at 32°, and one set at 37° for 3 days. (If 20° and 32° incubators are not available, prepare duplicate sets of plates and incubate one set at 37° and the other at room temp. Give approximate room temp. range during incubation period.) Count plates with aid of Quebec colony counter, if available. Express final results as numbers of viable microorganisms per gram of egg material.

5

## INCIDENCE OF COLIFORM GROUP

(a) *Presumptive test*.—Inoculate 1.0 ml portions from suitable dilutions (1-10 to 1-100,000,000) of egg material into fermentation tubes of lactose broth. Incubate at 37° for 24-48 hours. Streak eosin methylene blue or Endo agar plates from all lactose broth cultures showing gas production. Incubate plates at 37° for 24-48 hours. Examine plates of differential media for colonies of microorganisms of coliform group. Record number of coliform bacteria per gram of egg material as reciprocal of highest dilution showing positive confirmation on differential media.

(b) *Completed test*.—Inoculate from colonies of coliform types of bacteria appearing on differential agar plates to nutrient agar slants. Incubate at 37° for 24 hours. Purify cultures for further study. Obtain biochemical reactions of purified cultures by following tests:

*Kovac's test*: Indol production;

*Methyl red (M.R.) and Voges Proskauer (V.P.) tests*;

*Koser's sodium citrate test*: Utilization of sodium citrate as sole source of carbon.

NOTE: Follow procedure recommended in Standard Methods of Water Analysis, 8th ed., 1936, of American Public Health Association for Biochemical Reactions.

6

## INCIDENCE OF HEMOLYTIC STAPHYLOCOCCI AND STREPTOCOCCI

Inoculate Petri plates with 1 ml portions of all dilutions from 1-100 to 1-1,000,000. Pour plates with veal-infusion agar containing 6% of defibrinated horse, sheep, or rabbit blood (0.6 ml of blood per 10 ml of media). Cool agar to 40° and add blood just prior to pouring plates. Incubate plates for 24 hours at 37°. Express final results as numbers per gram of egg material.

7

## TESTS FOR PUTREFACTIVE ANAEROBIC TYPES OF MICROORGANISMS

Exhaust tubes of Holman's cooked meat media, 11(d), before use, by heating in streaming steam for 15 min., followed by rapid cooling in water bath. Inoculate tubes of exhausted meat media with 1 ml portions of all dilutions from 1-10 to 1-100,000. Incubate inoculated tubes at 37° for 3-4 days. (Putrefactive anaerobes are evidenced by evolution of gas and digestion of meat.) Confirm cultures by microscopic examination of smears stained with Gram's stain. Record numbers of putrefactive anaerobes per gram of egg material as reciprocal of highest dilution showing positive anaerobic growth.

8

## TESTS FOR FUNGI

Inoculate Petri plates with 1 ml portions of all serial dilutions from 1-10 to 1-100,000. Pour inoculated plates with malt agar previously cooled to 40-45°. Incubate plates for 3 days at 20°. Express final results as numbers of fungi per gram of egg material.

9

## DIRECT MICROSCOPIC COUNTS

Place 0.01 ml of the 1-10 or 1-100 dilutions of egg material on clean microscope slide and spread over area of 1 sq. cm. Permit smear preparation to dry on level surface at 30-40°. Proceed as directed in Standard Methods for the Examination of Dairy Products, 7th Ed., 1939, of the American Public Health Association. Multiply total count by 10 or 100, since the original smear preparation was made from a 1-10 or 1-100 dilution, in order to obtain total numbers of bacteria per gram of egg material.

## CULTURE MEDIA

10

## STANDARD METHODS MEDIA

Prepare following media as recommended in Standard Methods of Water Analysis, 8th Ed., 1936, of the American Public Health Association: Nutrient agar, Levine's eosin methylene blue agar, Endo agar, tryptophane broth, lactose broth, methyl red—Voges Proskauer peptone medium, and Koser's sodium citrate medium.

11

## OTHER MEDIA

(a) *Malt agar*.—Malt extract (bacto-dehydrated), 1.5% = 15.0 g; agar, 2.0% = 20.0 g; and H<sub>2</sub>O, 1000 ml.

Sterilize mixture at 15 lbs. pressure for 20 min. Final pH = 4.6±.

(b) *Physiological salt soln.*—NaCl, C.P., 0.85% = 8.5 g; and H<sub>2</sub>O, 1000 ml.

Sterilize mixture at 15 lbs. pressure for 15 min.

(c) *Veal infusion agar*.—Ground lean veal, 500.0 g, and H<sub>2</sub>O, 1000 ml.

Infuse overnight in refrigerator and strain thru cheese cloth without pressure. Make up to original volume with H<sub>2</sub>O and skim off any fat. Steam in Arnold 30 min. and filter thru paper. Add peptone (Difco), 1.0% or 10.0 g; NaCl, 0.5% or 5.0 g; and agar, 1.5% or 15.0 g.

Steam in Arnold to dissolve ingredients. Adjust reaction to pH 7.6 and steam in Arnold 15 min. Filter thru Büchner funnel with paper pulp mat, by aid of suction. Use egg albumin for clarification when necessary. Add fresh white of 1 egg previously beaten with 50 ml of the medium or its equivalent in desiccated egg white (1.5 g) to each liter of the medium before adjustment of reaction and after cooling to 50°. Shake thoroly to insure solution of egg white. Allow to stand 20 min. Heat in Arnold for 15 min. to coagulate egg white. Shake vigorously and reheat. Filter. Adjust reaction to pH 7.6. Steam in Arnold 15 min. Filter. Distribute 10 ml quantities into test tubes or 80 ml quantities into bottles. Sterilize at 15 lbs. pressure (121°) for 20 min. Final pH 7.4. For hemolytic tests cool melted agar to 40° and add 6% of defibrinated horse, sheep, or rabbit blood prior to pouring plates (0.6 ml of blood per 10 ml of media).

(d) *Holman's cooked meat medium (alkaline)*.—Ground fresh lean beef, 500.0 g; H<sub>2</sub>O, 1000 ml; peptone (Bacto), 5.0 g; and NaCl, C.P., 5.0 g.

Add beef to the H<sub>2</sub>O and infuse overnight in refrigerator. Skim off fat. Strain thru several layers of cheese cloth and press out broth, retaining meat press cake. Make up to original volume of 1 liter. Add peptone and heat in Arnold 10 min. Filter and add salt. Add normal NaOH until alkaline to phenolphthalein. Heat in Arnold 15 min. to clear, and filter. Distribute pressed-out beef remaining from infusion into test tubes (150×20 mm), ca 2 g into each tube, and add 10 ml of the clear alkaline broth. Sterilize in autoclave at 15 lbs. pressure (121°) for 20 min. Final reaction pH 7.2-7.4. Prior to use, boil tubed medium at least 10 min. to expel adsorbed oxygen and cool promptly in water bath.

### DETECTING AND ESTIMATING NUMBERS OF THERMOPHILIC BACTERIA IN SUGAR<sup>2</sup>

(Sugar, both beet and cane, may carry spores of all three groups of thermophilic bacteria that are important as spoilage agents in non-acid canned foods, i.e., flat sour bacteria (*Bacillus stearotherophilus*), thermophilic anaerobes, not producing hydrogen sulfide (*Clostridium thermosaccharolyticum*), and sulfide spoilage bacteria (*Cl. nigrificans*). These bacteria are economically the most important causes of spoilage thru understerilization of non-acid canned vegetables.)

#### 12

##### SAMPLING

Take  $\frac{1}{2}$  lb. samples from each of 5 bags or barrels of shipment or of lot in question. Send these samples to laboratory in clean sealed cans, or other appropriate containers.

(The adequacy of this sampling will vary in relation to size of shipment or lot, and if there is any significant variability in the shipment, this fact will become evident, in the majority of cases, thru individual tests on 5 samples.)

#### 13

##### PREPARATION OF SAMPLE

Place 20 g of sugar in a sterile 150 ml Erlenmeyer flask marked to indicate a volume of 100 ml. Add sterile H<sub>2</sub>O to the 100 ml mark. Bring rapidly to boiling, and boil 5 min. Replace evaporation with sterile H<sub>2</sub>O.

#### 14

##### CULTURE MEDIA

(a) *Dextrose tryptone agar*.—For use in detection of flat sour bacteria.

This medium is prepared as a standardized, dehydrated medium and is marketed under the name of Bacto-dextrose Tryptone Agar by the Difco Laboratories, Inc., Detroit, Mich. Because of its standardization, its use in this form is recommended. It may, however, be prepared according to following formula: Tryptone, 10 g; dextrose, 5 g; agar, 15 g; bromocresol purple, 0.04 g; and H<sub>2</sub>O, 1000 ml.

(b) *Liver broth*.—For detection of thermophilic anaerobes not producing H<sub>2</sub>S (*Cl. thermosaccharolyticum*), putrefactive anaerobes, and other mesophilic anaerobes.

Mix chopped beef liver with H<sub>2</sub>O in proportion of 500 g to 1000 ml. Boil mixture slowly 1 hour, adjust to ca pH 7.0, and boil an additional 10 min. Then press boiled material thru cheese cloth and make liquid to 1000 ml. To broth add 10 g of peptone and 1 g of dipotassium phosphate. Adjust reaction to pH 7.0. In tubing, introduce  $\frac{1}{2}$ -1" of previously boiled ground beef liver into the tube.

Before using, unless it is freshly prepared, exhaust medium by subjecting to streaming steam at least 20 min., and after inoculation, stratify it with 2-2 $\frac{1}{2}$ " layer of plain nutrient agar (common formula), which has been cooled to 50°.

(c) *Sulfite agar (modified)*.—For detection of thermophilic anaerobes producing H<sub>2</sub>S (*Cl. nigrificans*).

Prepare according to following formula: Tryptone, 10 g; Na<sub>2</sub>SO<sub>3</sub>, 1 g; agar, 20 g; and H<sub>2</sub>O, 1000 ml.

At time of tubing place a clean iron strip or nail in tube. No adjustment in reaction is necessary. Prepare medium at frequent intervals (1 week), and if Na<sub>2</sub>SO<sub>3</sub> is used in soln also prepare it at frequent intervals (1 week).

##### CULTURE TECHNIC

#### 15

##### DETECTION OF FLAT SOUR SPORES

Into each of 5 Petri dishes pipet 2 ml of the boiled sugar soln. Cover, and mix inoculum with the dextrose tryptone agar. Incubate plates at 55° for 36-48 hours, and to prevent drying of the agar, humidify the incubator. The combined count from the 5 plates represents the number of spores in 2 g of original sugar. Multiply this count by 5 to express results in terms of number of spores per 10 g of sugar

These colonies are characteristic. A colony is round, measures 2-5 mm in diameter, presents a typical opaque central "spot," and is usually surrounded by a yellow halo in a field of purple. This halo may be insignificant, or missing, where certain low acid-producing types are concerned, or where the plate is so thickly seeded that the entire plate takes on a yellow tinge. The typical subsurface colonies are rather compact and may approach the "pin point" condition.

If there is doubt as to the identity of the sub-surface colonies, a decision can usually be made after observing the nature of the surface colonies. If they show reasonable purity of flora, it is safe for practical purposes to assume that the subsurface colonies have been formed by similar bacterial groups. It is emphasized that where the plate is heavily seeded, there may be loss of accuracy as regards counts, and colony structure and size may be atypical. If plates are so heavily seeded as to make counting impracticable, a second sample of the sugar may be plated, dilutions of the original solution being used.

Whether atypical subsurface colonies are flat sour organisms may often be determined by streaking from the colonies to agar plates so that their surface characteristics may be noted.

No immediate significance is attached to the presence of "non-spoilage" thermophiles; i.e., aerobic spore-formers, actinomycetes, etc., although when present in large numbers they carry significance with regard to the general bacteriological quality of the sugar. The total thermophilic spore count may be obtained from the dextrose tryptone agar plates.

## 16

DETECTION OF THERMOPHILIC ANAEROBES NOT PRODUCING  $H_2S$ 

(Under the conditions stated, thermophilic anaerobes are manifest thru the splitting of agar and the presence of acid. At times a cheesy odor is noted. The method is considered suitable as a qualitative test but quantitatively it provides only a means of rough estimation. The method does not permit expression of results in terms of numbers of spores per unit weight of sugar.)

Divide 20 ml of the sugar soln approximately equally among 6 liver broth tubes and stratify the liquid medium with plain nutrient or yeast water agar. After agar has solidified, preheat to 55° and incubate at that temp. for 72 hours.

## 17

DETECTION OF THERMOPHILIC ANAEROBES PRODUCING  $H_2S$ 

## (SULFIDE SPOILAGE ORGANISMS)

(In sulfite agar the sulfide spoilage organisms are detected thru the formation of characteristic blackened spherical areas. Due to the solubility of  $H_2S$  and its fixation by the iron, no gas is noted. Certain of the thermophilic anaerobes (not producing  $H_2S$ ), methods for the detection of which precede, give rise to relatively large quantities of  $H_2$ , which splits the agar and reduces the sulfite, thereby causing general blackening of the medium. This condition, however, is readily distinguishable from the restricted blackened areas mentioned previously. The blackened areas may be counted to obtain quantitative results.)

Divide 20 ml of the sugar soln approximately equally among 6 freshly exhausted tubes containing the sulfite agar. Incubate at 55° for 72 hours.

## 18

## REPORTING RESULTS

Report flat sour and sulfide spoilage results as number of spores per 10 g of sugar. Report thermophilic anaerobes (not producing  $H_2S$ ) as number of tubes positive and number negative in the following manner: +++ ---.

EXAMINATION OF CANNED VEGETABLES<sup>3</sup>

## 19

## SAMPLING

The procedure to be followed in the microbiological examination of canned vegetables is indicated by the purpose of the examination. Samples are commonly submitted to the laboratory for one of following three purposes:

1. Unspoiled samples—for direct bacteriological examination for sterility.
2. Unspoiled samples—for examination as to keeping quality.
3. Spoiled samples—for examination as to cause of spoilage.

The technic of examination for all three types of samples is similar with respect to treatment of container, removal of samples, and culture methods. Differences in treatment include the following:

(a) Before being cultured, unspoiled samples submitted for examination as to keeping quality should be incubated at 37.5° for 1 month. This time of incubation, necessarily an arbitrary matter, is considered the longest period that is practicable in the usual case. Anaerobes, which at times may remain dormant for many months, may escape detection, but usually the likelihood of spoilage in the product under ordinary commercial conditions of handling is indicated in this time. With canned vegetables it is frequently necessary to incubate at 55° in order to determine possibility of thermophilic spoilage in event of undercooling following processing. At 55° incubation for 10 days is recommended.

(b) Samples submitted for examination for cause of spoilage should be given direct microscopical examination in order to obtain general information regarding the bacterial flora. Ordinary laboratory stains, such as carbol fuchsin or gentian violet, are suitable in preparing mounts. The Gram stain is not recommended. A Gram negative result would be of little significance because of lack of knowledge regarding age of bacteria in spoiled material.

(a) *Physical examination and preparation of can.*—(1) Note and record all marks of identification, either embossed on can or appearing on label.

(2) Remove labels. Record any physical defects such as rustiness, pin-holing, dents, improper closure, or defective side seams. Plainly mark for inspection questionable points if can is to be pumped or given any other physical examination after it is opened.

(3) Clean container with soap and H<sub>2</sub>O; if it is greasy, it may be found helpful, especially at site of opening, to apply a suitable solvent, such as petroleum benzin, alcohol, or naphtha.

(4) For sterilization at site of opening, preferably grasp container in the hand and hold previously cleaned top in flame of a Bunsen burner, distributing heat with circular motion. Do not play burner down upon top of can because this will result in a concentration of heat at top, causing scorching of material, and it might lead to spurting of contents when opening is made. (Such sterilization also causes a release of vacuum in can, which will prevent any contamination that might result from an inrush of air when the opening is made.) When containers are badly swollen,<sup>4</sup> for safety preferably sterilize with bichloride of mercury (1+1000) for a few seconds, dry with sterile towel, and sample without flaming; or thoroly clean cans with 60% alcohol. Whichever later treatment is used, first thoroly cleanse cans with soap and H<sub>2</sub>O, as it is possible that neither the bichloride nor the alcohol treatment would insure complete destruction of spore contaminants in the time that elapses between the sterilization treatment and the opening of the container.

(b) *Removal of sample.*—(1) *Opening of container.*—After flaming, or otherwise sterilizing the point of opening, make aperture with appropriate type of opener, which has also previously been sterilized by flaming. (Openers of spiral or circular type are preferred.) With liquid products, puncture an opening with a sharpened instrument of appropriate diameter.<sup>5</sup>

(2) *Inoculum.*—Determine type of instrument to be used for removal of inoculum by character of the food under examination. Sample liquid or semi-liquid food products with sterile untapered pipets or inverted 10 ml pipets. Sample solid material with sterile cork borers or brass sampling tubes after they have been wrapped in paper and sterilized 30 min. at 15 lbs. pressure in autoclave. Stopper samplers with cotton plugs, and force solid material into culture tubes by means of sterile glass rod or some similar device. Take a sample of at least 15 g of food material, which

may be cultured directly into 1 culture tube or flask, but preferably into at least 3 culture vessels. If the material is solid, mix it with sterile  $H_2O$  as preliminary step to inoculation.

## 20

CULTURE MEDIA<sup>6</sup>

For routine culture purposes, dextrose tryptone agar and liver broth are suitable for the detection of those organisms that are the principal cause of spoilage in non-acid canned vegetables, as well as for the detection of less important organisms occasionally encountered, and use of these media is recommended. Where special examination is made for putrefactive anaerobes, beef heart peptic digest is recommended to supplement liver broth cultures. Corn-liver medium should supplement liver broth in special search for thermophilic anaerobes or in their study after isolation. Sulfite agar is useful only when indicated by a type of spoilage characterized by the presence of  $H_2S$ .

(a) *Dextrose tryptone agar*.—See 14(a).

This medium is used principally for the isolation of flat sour bacteria from original products or from enrichment cultures. It is also suitable for the isolation of other aerobic or facultative anaerobic bacteria, such as may be encountered in non-acid canned foods. For flat sour bacteria, incubation is usually at 55°.

(b) *Liver broth*.—See 14(b).

In this medium, thermophilic anaerobes are evident thru the splitting of agar and the presence of acid. At times, a cheesy odor is noted. When incubation is at 37°, the presence of putrefactive anaerobes becomes apparent thru splitting of the agar, resulting from gas production, and the presence of a putrid odor.

(c) *Beef heart peptic digest broth*<sup>7</sup>.—Used principally for detection of putrefactive anaerobes and their cultural study.

This medium is difficult to prepare, but if any intensive study of putrefactive spoilage is made, it is regarded as a valuable medium.

(1) Slowly heat to boiling 1000 g of finely ground, fat-free heart, and 1000 ml of tap  $H_2O$ , and adjust to reaction of pH 8.0–8.2. Cool, and carefully skim off layer of fat that floats on the cold medium. To each liter of beef heart mash, add 2 liters of peptic digest broth, (2). Adjust reaction to pH 7.2–7.4.

(2) Wash clean and mince finely 5 or more large pig stomachs. Mince an equal amount of clean pig or beef liver. Mix in following proportions: Minced pig stomachs, 400 g; minced liver, 400 g; HCl, 40 g; and tap  $H_2O$  at 50°, 4000 g.

Keep mixture in glass or porcelain receptacles for 18–24 hours. Make biuret and also tryptophan tests. When both reactions are positive, the digest is green-yellowish and contains little undigested debris. Transfer digest to large bottles and steam 10 min. at 100° to stop digestion. Strain digest thru cotton or preferably store overnight in ice chest and decant after 24 hours. Warm decanted digest to 70° and neutralize with  $Na_2CO_3$  (twice normal solution) to litmus at this temp. Filter desired quantity, add 0.2% dipotassium phosphate; adjust to pH 7.4, and mix with beef heart mash. Adjust final reaction and sterilize 1 hour at 18 lbs. pressure. Incubate for 5 days and repeat same sterilization for 1 hour at 18 lbs. pressure.

Before inoculation, exhaust as directed in 14(b). After inoculation, stratify with sterile vaseline.

(d) *Corn-liver medium*<sup>8</sup>.—For detection of thermophilic anaerobes not producing  $H_2S$  (*Cl. thermosaccharolyticum*), putrefactive anaerobes, and other mesophilic anaerobes.

For a time it is suggested that this medium be used in conjunction with another anaerobic medium such as liver broth, in order that comparative data may be obtained.

Steam 1 or 2% liver (tissue from liver infusion medium dried at 55 to 60° and



finely ground) and 5% corn meal for 1 hour, cool, and tube. Carefully sterilize the resulting, rather viscous medium by autoclaving 2 hours at 15–17 lbs. pressure. If pressure is reduced slowly after sterilization, short (6") tubes may be used without blowing the plugs. This medium need not be steamed just before using, requires no seal nor incubation in an anaerobic jar, and satisfactory results may be obtained with 2–5 cm depth of medium.

Positive cultures are recognized by the appearance of gas with or without digestion of the medium. There may also be a measurable change in the reaction of the medium.

(e) *Sulfile agar*.—For detection of thermophilic anaerobes producing  $H_2S$  (*Cl. nigrificans*).

Sulfide spoilage organisms are detected thru formation of characteristic blackened spherical areas. Usually no gas forms. The presence of gas coupled with general blackening of medium indicates presence of thermophilic anaerobes not of sulfide spoilage group. This darkening results from reduction by hydrogen gas.

Use following formula: Water, 1 liter; tryptone, 10 g;  $Na_2SO_3$ , 1 g; and agar, 20 g.

At time of tubing place a clean iron strip or nail in the tube. No adjustment in reaction is necessary.

## 21

### ROUTINE EXAMINATIONS

Incubate original cultures at 37.5° for 48–72 hours.

### SELECTED REFERENCES

<sup>1</sup> J. Assoc. Official Agr. Chem., **22**, 625 (1939).

<sup>2</sup> Ibid., **19**, 439 (1936); **21**, 457 (1938).

<sup>3</sup> Ibid., **21**, 452 (1938).

<sup>4</sup> Ibid., **19**, 430 (1936).

<sup>5</sup> Ibid., 431.

<sup>6</sup> Ibid., 433.

<sup>7</sup> J. Infectious Diseases, **31**, 505 (1922).

<sup>8</sup> J. Bact., **28**, 267 (1934).

# XLI. MICROCHEMICAL METHODS

1

## METHOXYL AND ETHOXYL GROUPS—TENTATIVE

### REAGENTS

(a) *Acetic acid-potassium acetate soln.*—Dissolve 10 g of K acetate in sufficient glacial acetic acid to make 100 ml of soln.

(b) *Sodium acetate soln.*—Dissolve 25 g of crystalline Na acetate in sufficient  $H_2O$  to make 100 ml of soln.

(c) *Approximately 0.05 N thio-sulfate soln.*—Boil 2.5 liters of  $H_2O$  until  $\frac{1}{2}$  has evaporated, cool to ca  $75^\circ$ , and then add the necessary thiosulfate and 20 ml of amyl alcohol (by-product from alcoholic fermentation). Allow to cool and standardize against a standard  $KIO_3$  soln.

### 2 DETERMINATION

To 5 ml of the K acetate soln, add 0.2 ml of  $Br_2$ , and place  $\frac{2}{3}$  of this liquid in receiver C and the remainder in D (Fig. 61). Then weigh ca 20 mg of substance upon a tared piece of cigarette paper ( $15 \times 25$  mm) and place both paper and contents in bottom of boiling flask A, together with a boiling rod. (A glass tube ca 60 mm long, 3.5 mm o.s. diameter with a 1 mm bore. It is sealed at one end and also closed ca 10 mm from the other. The open end is fire polished. When this rod is placed in flask with open end down it will cause uniform boiling indefinitely if sufficient heat is constantly applied to flask.) Add 2.5 ml of melted phenol from a wide-tipped pipet and 5 ml of HI and connect flask to remainder of apparatus, which consists of the trap (B), containing a little  $H_2O$ , and the receivers C and D. Pass  $CO_2$  thru apparatus from capillary side arm of boiling flask at uniform rate of 15 ml per min., and boil liquid by means of a mantled micro burner at such rate that the vapors of the boiling liquid rise about half way in the air condenser. Continue boiling 30–60 min. (If the type of substance is known to require only 30 min., this period

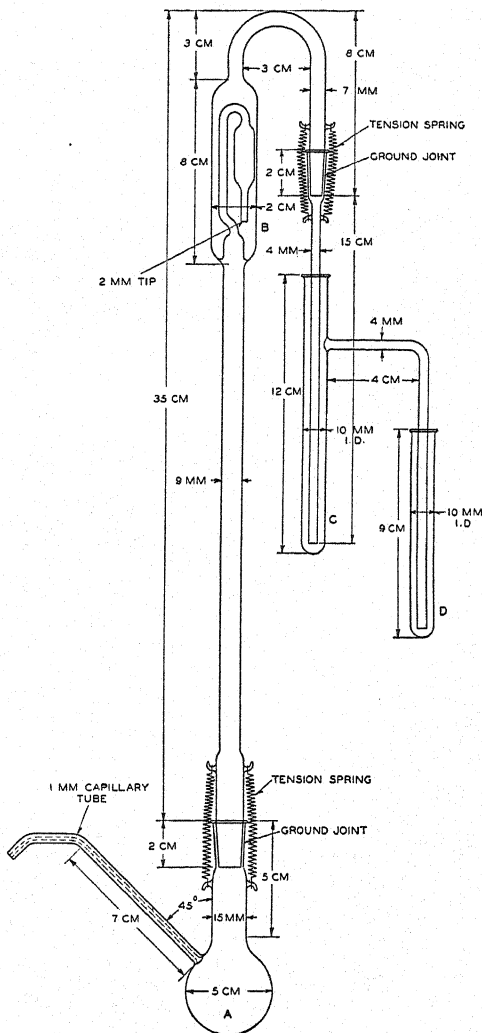


FIG. 61.—SEMI-MICRO ZEISEL METHOXYL APPARATUS

should be used, but for material about which such information is lacking an hour should be used as a general procedure.) Disconnect apparatus and wash contents of receivers into a 250 ml Erlenmeyer flask containing 5 ml of Na acetate soln. Adjust volume of liquid to 100 ml and reduce excess  $\text{Br}_2$  with formic acid (ca 15 drops is sufficient).

Remove any  $\text{Br}_2$  vapor in flask by drawing air over liquid from vacuum line or by blowing air over liquid, then add 0.5 g of KI and 5 ml of 10%  $\text{H}_2\text{SO}_4$  soln. Titrate liberated I with the thiosulfate soln, using starch as indicator.

Obtain blank on all reagents by making a determination without a sample and subtract this from quantity of thiosulfate soln used when sample was present. 1 ml of 0.05 *N* thiosulfate = 0.2586 mg of methoxyl ( $\text{OCH}_3$ ).

The same procedure applies to ethoxyl groups. 1 ml of 0.05 *N* thiosulfate = 0.3754 mg of ethoxyl ( $\text{OC}_2\text{H}_5$ ). For determination of higher alkoxyls the air condenser and scrubber may be surrounded by a water jacket heated to necessary temperature.

### 3

#### OTHER MICROCHEMICAL METHODS

Other microchemical methods that have been adopted will be found in the chapters to which they apply.

#### SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 15, 136 (1932); 22, 100, 622 (1939).

## XLII. STANDARD SOLUTIONS

### SODIUM HYDROXIDE<sup>1</sup>—OFFICIAL

1

#### APPARATUS

The buret and pipet used should be Bureau of Standards calibrated or should be calibrated by the analyst. Automatic burets should have all exits to the air protected from CO<sub>2</sub> contamination by suitable guard tubes containing soda-lime. All containers should be of alkali-resisting glass.

2

#### REAGENTS

(a) *Carbonate-free H<sub>2</sub>O*.—Prepare by one of the following methods: (1) Boil distilled H<sub>2</sub>O 20 min. and cool with soda-lime protection; (2) bubble air, freed from CO<sub>2</sub> by passing thru a tower of soda-lime, thru distilled H<sub>2</sub>O for 12 hours.

(b) *1+1 alkali*.—To one part of NaOH (reagent quality containing less than 5% Na<sub>2</sub>CO<sub>3</sub>) in a flask add one part of distilled H<sub>2</sub>O and swirl until solution is complete. Close with a rubber stopper. Set aside until Na<sub>2</sub>CO<sub>3</sub> has settled, leaving a perfectly clear liquid (about 10 days).

(c) *Potassium acid phthalate*.—U. S. Bureau of Standards Sample Standard for Acidimetry. Dry for 2 hours at 120°. Cool in a desiccator containing H<sub>2</sub>SO<sub>4</sub>.

(d) *Phenolphthalein indicator*.—1.0 g in 100 ml of alcohol.

3

#### PREPARATION OF STANDARD SOLUTION

The following table gives the approximate amount of 1+1 alkali necessary to make 10 liters of standard soln:

<i>Approx. normality</i>	<i>1+1 alkali to be diluted to 10 liters (ml)</i>
0.01	5.4
0.02	10.8
0.10	54.0
0.50	270.0
1.0	540.0

Add the required amount of 1+1 alkali to 10 liters of CO<sub>2</sub>-free H<sub>2</sub>O. Check the normality, which should be slightly strong, as directed under 4, and adjust to desired strength by the following formula:  $V_1 = V_2 \times N_2 / N_1$ , where  $N_2$  and  $V_2$  represent the normality and volume of stock soln, respectively, and  $V_1$  the volume to which the stock soln should be diluted to obtain the desired normality,  $N_1$ . Determine the exact strength of the final soln as directed under 4.

4

#### STANDARDIZATION<sup>1</sup>

Accurately weigh sufficient dried acid potassium phthalate to titrate approximately 40 ml and transfer to a 300 ml flask that has been swept free from CO<sub>2</sub>. Add 50 ml of cool CO<sub>2</sub>-free H<sub>2</sub>O. Stopper the flask and swirl gently until the sample is dissolved. Add 3 drops of the phenolphthalein indicator, 2(d), and titrate with the soln that is being standardized.

Calculate the normality ( $N$ ) of the standard soln by the following formula:

$$N = \frac{\text{g potassium acid phthalate}}{\text{ml NaOH} \times 204.136/1000}$$

(The normality value is exact only when phenolphthalein is used as an indicator.)

## HYDROCHLORIC ACID—OFFICIAL, FIRST ACTION

5

## PREPARATION OF STANDARD SOLUTIONS

The following table gives the approximate amount of HCl (reagent quality, 35–37% HCl) necessary to make 10 liters of standard solns:

<i>Approx. normality</i>	<i>HCl to be diluted to 10 liters (ml)</i>
0.01	8.9
0.02	17.8
0.10	89.0
0.50	445.0
1.0	890.0

6

## STANDARDIZATION

Titrate 40 ml against a standard alkali soln of approximately the same strength as the acid being standardized as directed under 4, using phenolphthalein as an indicator, 2(d). Determine the normality by the following formula:

$$N = \frac{\text{ml standard alkali} \times \text{normality of alkali}}{\text{ml HCl}}$$

If stronger than desired, dilute the soln to a definite normality value by the following formula:

$$V_1 = \frac{V_2 \times N_2}{N_1}, \text{ where}$$

$N_2$  and  $V_2$  represent the normality and volume of stock soln, respectively, and  $V_1$  represents the volume to which the stock soln should be diluted to obtain the desired normality,  $N_1$ .

Check the exact strength of the final soln by titration as directed above. The normality will be exact only if the same indicator is used in a determination and in the standardization.

If the standard acid soln is to be used with methyl orange as an indicator, determine a correction for the volume of acid required to pass from the end point of phenolphthalein to that of methyl orange. Add 3 drops of a 1% soln of phenolphthalein to 100 ml of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , and then add sufficient alkali soln to give an end point with phenolphthalein. Disregard the quantity of alkali soln added and take the buret readings from this point. Add 3 drops of a 0.02% soln of methyl orange and sufficient 0.1  $N$  acid to produce the pink color of methyl orange. Titrate back with 0.1  $N$  alkali soln to the same end point taken in the usual titration (preferably  $\text{pH} = 4.2$ ). Buffered solns of 3.8, 4.0, and 4.2  $\text{pH}$  are useful in accurately determining the methyl orange end point. They may be prepared as follows:<sup>2</sup>

$\text{pH} = 3.8$ , 2.041 g KH phthalate + 5.30 ml 0.1  $N$  HCl. Dilute to 200 ml.

$\text{pH} = 4.0$ , 2.041 g KH phthalate + 0.80 ml 0.1  $N$  NaOH. Dilute to 200 ml.

$\text{pH} = 4.2$ , 2.041 g KH phthalate + 7.30 ml 0.1  $N$  NaOH. Dilute to 200 ml.

If the acid and alkali solns are equivalent, the quantity of acid – the quantity of alkali soln = the quantity of acid required to pass from the phenolphthalein end point to that of methyl orange.

## STANDARDIZATION OF ACID SOLUTIONS

*I. With Borax<sup>3</sup>—Official*

7

## REAGENTS

(a) *Methyl red indicator*.—Dissolve 100 mg of methyl red in 60 ml of alcohol and dilute with  $\text{H}_2\text{O}$  to 100 ml.

(b) *Sodium borate*.—U.S.P. quality or better; soln of 5 g of salt in 95 ml of warm H<sub>2</sub>O should pass the following purity tests:

(1) *Insoluble impurities*.—Should be clear and colorless.

(2) *Chloride*.—20 ml must not give an opalescence with HNO<sub>3</sub> and AgNO<sub>3</sub> that is stronger than 20 ml of a Cl soln that has a strength of 5 mg of Cl per liter.

(3) *Sulfate*.—20 ml should give no precipitate with acetic acid and BaCl<sub>2</sub> after standing 30 min.

(4) *Calcium*.—20 ml of the hot soln should give no turbidity with NH<sub>4</sub> oxalate after cooling.

(5) *Magnesium*.—20 ml of soln must not give any microcrystalline precipitate with ammonia and phosphate after standing 24 hours.

(c) *Reference soln*.—Prepare a reference soln of boric acid, NaCl, and indicator corresponding to composition and volume of the soln at equivalence point. For use in determination of end point of a titration with 0.1 *N* acid, the reference soln should be 0.1 *M* in boric acid and 0.05 *M* in NaCl.

(d) *Standard borax*.—Saturate 300 ml of H<sub>2</sub>O at 55° (not higher) with borax (ca 45 g). Filter at this temp. thru folded filter into 500 ml Erlenmeyer flask. Cool filtrate to ca 10°, with continuous agitation during the crystallization. Decant supernatant liquid. Rinse precipitate once with 25 ml of cold H<sub>2</sub>O. Dissolve crystals in just enough H<sub>2</sub>O at temp. of 55° to insure complete soln (ca 200 ml). Re-crystallize by cooling to ca 10°, agitating the flask during crystallization. Filter crystals onto small Büchner funnel with suction. Wash precipitate once with 25 ml of ice-cold H<sub>2</sub>O. Dry crystals<sup>4</sup> by washing with two 20 ml portions of alcohol, drying after each washing with suction. Follow with two successive 20 ml portions of U.S.P. ether. (Both the alcohol and ether just prior to use should be freed from any possible reacting acids by shaking each vigorously with 2 or 3 g of the pure, dry borax, then filtering.) After spreading on a watch-glass, immediately place the dried borax in a desiccator over a soln saturated with respect to both sugar and salt and allow to remain at least 24 hours before using. Then transfer the pure borax into container that has ground-glass stopper and store in desiccator when not in use (stable under these conditions for 1 year).

## 8

## STANDARDIZATION

Accurately weigh sufficient of the standard borax to titrate ca 40 ml and transfer to a 300 ml flask. Add 40 ml of CO<sub>2</sub>-free H<sub>2</sub>O and stopper the flask. Swirl gently until the sample is in soln. Add 4 drops of the methyl red indicator and titrate with the soln that is being standardized to the equivalence point as indicated by the reference soln. Calculate the normality (*N*) of the standard soln by the following formula:

$$N = \frac{\text{g of Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}}{\text{ml of acid} \times 190.72/1000}.$$

## II. With Sodium Carbonate—Official

## 9

## REAGENTS

(a) *Methyl orange indicator*.—0.1% in H<sub>2</sub>O.

(b) *Sodium bicarbonate*.—C.P. Should pass the following tests for purity:

(1) *Chloride*.—0.5 g of NaHCO<sub>3</sub> dissolved in 10 ml of 2 *N* HNO<sub>3</sub> (free of Cl) must give no opalescence with AgNO<sub>3</sub>.

(2) *Sulfate*.—0.5 g of NaHCO<sub>3</sub> in 10 ml of 2 *N* acetic acid should give no turbidity or separation of BaSO<sub>4</sub> after the addition of BaCl<sub>2</sub> and standing 15 min.

(c) *Reference soln.*—80 ml of CO<sub>2</sub>-free H<sub>2</sub>O with 3 or 4 drops of methyl orange indicator.

(d) *Anhydrous sodium carbonate.*<sup>5</sup>—Heat 250 ml of H<sub>2</sub>O to 80° and add NaHCO<sub>3</sub>, stirring until no more dissolves. Then filter the soln thru a folded filter (the use of a hot water funnel is desirable) into an Erlenmeyer flask. Cool the filtrate to about 10° with constant swirling during crystallization. The fine crystalline trona and bicarbonate that separates out has the approximate composition: Na<sub>2</sub>CO<sub>3</sub> · NaHCO<sub>3</sub> · 2H<sub>2</sub>O. Pour off the mother liquor. Drain the crystals by suction and wash once with cold H<sub>2</sub>O.

Transfer the precipitate, being careful not to include any fibers of filter paper, into a large flat-bottomed dish. Heat in an electric oven or furnace with a pyrometer control at a temp. of 290° for 1 hour. Stir the contents occasionally with a Pt wire. After heating, cool the Pt dish and contents in a desiccator. Store the anhydrous Na<sub>2</sub>CO<sub>3</sub> in a container having a ground-glass stopper in a desiccator containing a good desiccant. Dry the salt at 120° just before using.

## 10

## STANDARDIZATION

Accurately weigh sufficient anhydrous Na<sub>2</sub>CO<sub>3</sub> to titrate ca 40 ml and transfer to a 300 ml Erlenmeyer flask. Add 40 ml of H<sub>2</sub>O to dissolve the salt. Add 3 drops of the methyl orange indicator and titrate<sup>6</sup> until the color begins to deviate from the H<sub>2</sub>O tint (reference soln). (The equivalence point has not been reached.) Boil the soln gently 2 min., then cool. Titrate until the color is barely different from the H<sub>2</sub>O tint (of the indicator).

Calculate the normality (*N*) of the standard soln by the following formula:

$$N = \frac{\text{g of Na}_2\text{CO}_3}{\text{ml of acid} \times 53/1000}$$

## SULFURIC ACID—TENTATIVE

*I. Standard Borax Method*<sup>7</sup>

## 11

## PREPARATION OF STANDARD SOLUTION

The following table gives the approximate amount of H<sub>2</sub>SO<sub>4</sub> (reagent quality, ca 94% H<sub>2</sub>SO<sub>4</sub>) necessary to make 10 liters of standard solns:

<i>Approx. normality</i>	<i>H<sub>2</sub>SO<sub>4</sub> to be diluted to 10 liters (ml)</i>
0.01	2.8
0.02	5.7
0.10	28.4
0.50	141.8
1.0	283.5

## 12

## STANDARDIZATION.—See 7

## 13

*II. Specific Gravity Method*<sup>8</sup>

Dilute reagent quality, ca 94% H<sub>2</sub>SO<sub>4</sub>, with sufficient H<sub>2</sub>O to make a convenient quantity of ca 70% H<sub>2</sub>SO<sub>4</sub> soln. Determine sp. gr. in air at a convenient temp. (0–40°) as directed in XIV, 3, protecting soln from contact with the air. Calculate exact percentage of H<sub>2</sub>SO<sub>4</sub> by means of the equation—

$$P = S(85.87 + 0.05 T - 0.0004 t^2) - 69.82;$$

where *P* = percentage of H<sub>2</sub>SO<sub>4</sub>, by weight; and *S* = sp. gr. (in air) at *T*°, compared with H<sub>2</sub>O at *t*°.

Weigh  $W$  grams of the prepared acid containing  $P\%$   $H_2SO_4$  and dilute to  $n$  liters to make required exactly standard soln containing  $A$  grams of  $H_2SO_4$  per liter.  $W$  may be calculated by the equation—

$$W = nA \times \frac{100}{P}.$$

## POTASSIUM PERMANGANATE—OFFICIAL, FIRST ACTION

14

## PREPARATION OF SOLUTION

Dissolve slightly more than desired equivalent weight (3.2 g for 0.1  $N$ ) of  $KMnO_4$  (A.R. or C.P. grade) in 1 liter of  $H_2O$ . Boil soln 1 hour. Protect from dust and allow to stand overnight. Clean thoroly 15 cm glass funnel, perforated porcelain plate from a Caldwell crucible, and glass-stoppered bottle (preferably of brown glass) with warm  $H_2SO_4$ - $K_2Cr_2O_7$  soln. Digest asbestos for use in Gooch crucibles on steam bath 1 hour, with ca 0.1  $N$   $KMnO_4$  that has been acidified with a few drops of  $H_2SO_4$  (1+3). Allow to settle, decant, and replace with  $H_2O$ . Prepare glass funnel by placing porcelain plate in apex, make pad of asbestos ca 3 mm thick on plate, and wash free from acid. The pad should not be too tightly packed and only moderate suction applied. Insert stem of funnel into neck of bottle and filter the  $KMnO_4$  soln directly into bottle without aid of suction.

15

## STANDARDIZATION

Weigh 0.25–0.30 g of Bureau of Standards  $Na_2C_2O_4$  in sufficient  $H_2O$  to make soln ca 0.1  $N$ . Add 15 ml of 4  $N$   $H_2SO_4$  for each 50 ml of soln. Heat to 75–85° and titrate with the  $KMnO_4$ , maintaining this temp. thruout the titration. Add the  $KMnO_4$  slowly, especially at beginning, and wait each time until soln becomes colorless. Continue titration to end point, with continuous stirring. Correct for excess of permanganate used for the end point by matching color in another beaker containing same quantity of acid and hot  $H_2O$ .

Solutions more dilute than 0.1  $N$  may be prepared by diluting a stronger soln with  $H_2O$  that has been distilled over alkaline permanganate, using usual precautions to prevent contamination with organic matter.

## SELECTED REFERENCES

- <sup>1</sup> J. Assoc. Official Agr. Chem., 19, 107 (1936).
- <sup>2</sup> Clark, Determination of Hydrogen Ions, 3rd ed., p. 200 (1928).
- <sup>3</sup> Kolthoff, Volumetric Analysis, II, 93–96 (1929); J. Assoc. Official Agr. Chem., 22, 102, 563 (1939); Hurley, F. H., Ind. Eng. Chem., Anal. Ed., 8, 220 (1936); 9, 237 (1937).
- <sup>4</sup> Smith and Croad, Ind. Eng. Chem., Anal. Ed., 9, 141–2 (1937).
- <sup>5</sup> Kolthoff, Volumetric Analysis, II, 88 (1929).
- <sup>6</sup> Ibid., 86.
- <sup>7</sup> J. Assoc. Official Agr. Chem., 22, 563 (1939).
- <sup>8</sup> J. Chem. Soc., Trans., 57, 64–184 (1890); J. Soc. Chem. Ind., (1899) 1091.





### XLIII. REFERENCE TABLES\*

	PAGE
1 International Atomic Weights.....	655
2 Various Strength Solutions of the Common Acids, Alkalies, and Alcohol..	656
3 Degrees Brix, Specific Gravity, and Degrees Baumé of Sugar Solutions...	658
4 Temperature Corrections to Readings of Saccharimeters (Standard at 20°C).....	664
5 Domke's Table of Apparent Specific Gravity of Sucrose Solutions at 20°C.	665
6 Refractive Indices of Sucrose Solutions at 20°C.....	667
7 Table of Corrections for Determining Percentage of Sucrose in Sugar Solutions by Means of Either Abbé or Immersion Refractometer When Readings Are Made at Temperatures Other Than 20°C.....	669
8 Table for Determining Percentage of Sucrose in Sugar Solutions from Readings of the Zeiss Immersion Refractometer at 20°C.....	670
9 Munson and Walker's Table for Calculating Dextrose, Levulose, Invert Sugar, Lactose, Sucrose, and Maltose.....	671
10 Herzfeld's Table for Determining Invert Sugar in Raw Sugars.....	677
11 Meissl and Hiller's Factors for Determining Invert Sugar in Materials in Which, of the Total Sugars Present, More Than 1.5 per cent Is Invert Sugar and Less Than 98.5 per cent Is Sucrose.....	678
12 Wein's Table for the Determination of Maltose.....	679
13 Copper-Levulose Equivalents According to Jackson-Mathews' Modification of Nyns' Selective Method for Levulose.....	680
14 Allihn's Table for the Determination of Dextrose.....	681
15 Factors for 10 ml Soxhlet's Solution To Be Used in Connection with the Lane-Eynon General Volumetric Method.....	683
16 Factors for 25 ml Soxhlet's Solution To Be Used in Connection with the Lane-Eynon General Volumetric Method.....	684
17 Quisumbing and Thomas Table for Calculating Dextrose, Levulose, Invert Sugar, Lactose, and Maltose.....	685
18 Kröber's Table for the Determination of Pentoses and Pentosans.....	686
19 Percentages by Volume at 60°F of Ethyl Alcohol Corresponding to Apparent Specific Gravities at Various Temperatures.....	689
20 Alcohol Table for Calculating the Percentages of Alcohol by Volume at 15.56°C in Mixtures of Ethyl Alcohol and Water from Their Zeiss Immersion Refractometer Readings and Indices of Refraction at 17.5-25°C.....	708
21 Percentages by Weight Corresponding to Various Percentages by Volume at 15.56°C (60°F) in Mixtures of Ethyl Alcohol and Water.....	716
22 For Determining Added Water in Milk by Means of the Freezing-Point Depression (Based on Winter's Table).....	717
23 Density of Carbon Dioxide (Parr).....	719
24 Correction Factors for the Gasometric Determination of Carbon Dioxide	721
25 Progressive Accumulation of Radium Emanation.....	726

\* The Jackson-Mathews refractometric and densimetric tables for levulose are given in *J. Assoc. Official Agr. Chem.*, 15, 86 (1932).

1

*International atomic weights.*<sup>1</sup>  
1940

	SYMBOL	ATOMIC NUMBER	ATOMIC WEIGHT		SYMBOL	ATOMIC NUMBER	ATOMIC WEIGHT
Aluminum.....	Al	13	26.97	Molybdenum...	Mo	42	95.95
Antimony.....	Sb	51	121.76	Neodymium....	Nd	60	144.27
Argon.....	A	18	39.944	Neon.....	Ne	10	20.183
Arsenic.....	As	33	74.91	Nickel.....	Ni	28	58.69
Barium.....	Ba	56	137.36	Nitrogen.....	N	7	14.008
Beryllium.....	Be	4	9.02	Osmium.....	Os	76	190.2
Bismuth.....	Bi	83	209.00	Oxygen.....	O	8	16.0000
Boron.....	B	5	10.82	Palladium.....	Pd	46	106.7
Bromine.....	Br	35	79.916	Phosphorus.....	P	15	30.98
Cadmium.....	Cd	48	112.41	Platinum.....	Pt	78	195.23
Calcium.....	Ca	20	40.08	Potassium.....	K	19	39.096
Carbon.....	C	6	12.010	Praseodymium..	Pr	59	140.92
Cerium.....	Ce	58	140.13	Protactinium...	Pa	91	231
Cesium.....	Cs	55	132.91	Radium.....	Ra	88	226.05
Chlorine.....	Cl	17	35.457	Radon.....	Rn	86	222
Chromium.....	Cr	24	52.01	Rhenium.....	Re	75	186.31
Cobalt.....	Co	27	58.94	Rhodium.....	Rh	45	102.91
Columbium.....	Cb	41	92.91	Rubidium.....	Rb	37	85.48
Copper.....	Cu	29	63.57	Ruthenium.....	Ru	44	101.7
Dysprosium.....	Dy	66	162.46	Samarium.....	Sm	62	150.43
Erbium.....	Er	68	167.2	Scandium.....	Sc	21	45.10
Europium.....	Eu	63	152.0	Selenium.....	Se	34	78.96
Fluorine.....	F	9	19.00	Silicon.....	Si	14	28.06
Gadolinium.....	Gd	64	156.9	Silver.....	Ag	47	107.880
Gallium.....	Ga	31	69.72	Sodium.....	Na	11	22.997
Germanium.....	Ge	32	72.60	Strontium.....	Sr	38	87.63
Gold.....	Au	79	197.2	Sulfur.....	S	16	32.06
Hafnium.....	Hf	72	178.6	Tantalum.....	Ta	73	180.88
Helium.....	He	2	4.003	Tellurium.....	Te	52	127.61
Holmium.....	Ho	67	163.5	Terbium.....	Tb	65	159.2
Hydrogen.....	H	1	1.0080	Thallium.....	Tl	81	204.39
Indium.....	In	49	114.76	Thorium.....	Th	90	232.12
Iodine.....	I	53	126.92	Thulium.....	Tm	69	169.4
Iridium.....	Ir	77	193.1	Tin.....	Sn	50	118.70
Iron.....	Fe	26	55.85	Titanium.....	Ti	22	47.90
Krypton.....	Kr	36	83.7	Tungsten.....	W	74	183.92
Lanthanum.....	La	57	138.92	Uranium.....	U	92	238.07
Lead.....	Pb	82	207.21	Vanadium.....	V	23	50.95
Lithium.....	Li	3	6.940	Xenon.....	Xe	54	131.3
Lutecium.....	Lu	71	174.99	Ytterbium.....	Yb	70	173.04
Magnesium.....	Mg	12	24.32	Yttrium.....	Y	39	88.92
Manganese.....	Mn	25	54.93	Zinc.....	Zn	30	65.38
Mercury.....	Hg	80	200.61	Zirconium.....	Zr	40	91.22

<sup>1</sup> Taken from *J. Am. Chem. Soc.*, 62, 672 (1940).

Various strength solutions of the common acids, alkalies, and alcohol.<sup>1</sup>

2

(a) *Hydrochloric Acid Solns*: Specification requires not less than 35% HCl by weight. Sp. gr. = 1.1778 at 15°. Mix with water and make up to 1 liter.

HCl STRENGTH DESIRED	HYDROCHLORIC ACID REQUIRED		
GRAMS PER LITER	GRAMS	ML	
5	14.29	12.13	Normal solution
10	28.57	24.26	
15	42.85	36.39	
20	57.14	48.52	
36.46	104.17	88.45	
50	142.86	121.29	
100	285.71	242.58	
150	428.57	363.88	
200	571.43	485.17	Constant boiling Sp. gr. 1.125
222.6	636.00	539.99	
278.4	795.43	675.35	
300	857.14	727.75	

(b) *Sulfuric Acid Solns*: Specification requires not less than 94% H<sub>2</sub>SO<sub>4</sub> by weight. Sp. gr. = 1.835 at 15°. Pour acid into excess of water and make up to 1 liter.

H <sub>2</sub> SO <sub>4</sub> STRENGTH DESIRED	SULFURIC ACID REQUIRED		
GRAMS PER LITER	GRAMS	ML	
5	5.32	3.0	For crude fiber
12.5	13.29	7.2	
20	21.28	11.6	
30	31.91	17.4	
40	42.55	23.2	Normal solution
49	52.13	28.4	
100	106.38	58.0	
150	159.57	87.0	
250	265.96	144.9	
300	319.15	173.9	
400	425.53	231.9	

(c) *Nitric Acid Solns*: Specification requires not less than 68% HNO<sub>3</sub> by weight. Sp. gr. = 1.4146 at 15°. 1 ml of concentrated HNO<sub>3</sub> contains ca 0.96 g of HNO<sub>3</sub>. Mix with water and make up to 1 liter.

HNO <sub>3</sub> STRENGTH DESIRED	NITRIC ACID REQUIRED	
GRAMS PER LITER	GRAMS	ML
5	7.35	5.2
10	14.71	10.4
20	29.41	20.8
30	44.12	31.2
40	58.82	41.6
50	73.53	52.0
63	92.65	65.5
70	102.94	72.8
100	147.06	104.0
150	220.59	156.0
200	294.12	207.9
300	441.18	312.9

<sup>1</sup> Prepared by G. C. Spencer and H. J. Fisher.

## 2 Various strength solutions of common acids, alkalies, and alcohol.—Concluded.

(d) *Ammonia Solns*: Specification requires not less than 27%  $\text{NH}_3$  by weight. Sp. gr. = 0.9. Mix and make to 1 liter.

NH <sub>3</sub> STRENGTH DESIRED	REAGENT AMMONIA REQUIRED	
	GRAMS	ML
5	18.52	20.6
10	37.04	41.1
15	55.55	61.7
20	74.07	82.3
25	92.59	102.9
50	185.18	205.8
75	277.77	308.6
100	370.37	411.5
150	555.55	617.3
200	740.74	823.0

(e) *Sodium Hydroxide Solns*: Specification requires 95% of NaOH in sticks of caustic soda. Dissolve and dilute to 1 liter.

NaOH STRENGTH DESIRED	SODIUM HYDROXIDE REQUIRED	
	GRAMS	
12.5	13.16	For crude fiber  Normal solution
30	31.58	
40	42.11	
50	52.63	
75	78.95	
100	105.26	
150	157.89	
200	210.53	
250	263.16	
300	315.79	

(f) *Alcoholic Solns*<sup>1</sup>: Specification requires 95%  $\text{C}_2\text{H}_5\text{OH}$  by volume. Sp. gr. = 0.810 at 25°. Mix and make to 1 liter.

ALCOHOL STRENGTH DESIRED	ALCOHOL REQUIRED	
	GRAMS	ML
50	42.63	52.6
100	85.26	105.3
150	127.89	157.9
200	170.52	210.5
250	213.16	263.2
300	255.78	315.9
400	341.04	421.1
500	426.32 (proof)	526.3
700	596.84	736.8

<sup>1</sup> Alcohol of any desired strength may be obtained by taking the number of ml of 95% alcohol equivalent to the desired strength and making the soln up to 95 ml. For example.—To obtain a solution of 70% alcohol, take 70 ml of 95% alcohol and dilute to 95 ml.

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions<sup>1</sup>*  
*(Plato's Table<sup>2</sup>).*

3

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
0.0	1.00000	0.998234	0.00	9.0	1.03586	1.034029	5.02
0.2	1.00078	0.999010	0.11	9.2	1.03668	1.034850	5.13
0.4	1.00155	0.999786	0.22	9.4	1.03750	1.035671	5.24
0.6	1.00233	1.000563	0.34	9.6	1.03833	1.036494	5.35
0.8	1.00311	1.001342	0.45	9.8	1.03915	1.037318	5.46
1.0	1.00389	1.002120	0.56	10.0	1.03998	1.038143	5.57
1.2	1.00467	1.002897	0.67	10.2	1.04081	1.038970	5.68
1.4	1.00545	1.003675	0.79	10.4	1.04164	1.039797	5.80
1.6	1.00623	1.004453	0.90	10.6	1.04247	1.040626	5.91
1.8	1.00701	1.005234	1.01	10.8	1.04330	1.041456	6.02
2.0	1.00779	1.006015	1.12	11.0	1.04413	1.042288	6.13
2.2	1.00858	1.006796	1.23	11.2	1.04497	1.043121	6.24
2.4	1.00936	1.007580	1.34	11.4	1.04580	1.043954	6.35
2.6	1.01015	1.008363	1.46	11.6	1.04664	1.044788	6.46
2.8	1.01093	1.009148	1.57	11.8	1.04747	1.045625	6.57
3.0	1.01172	1.009934	1.68	12.0	1.04831	1.046462	6.68
3.2	1.01251	1.010721	1.79	12.2	1.04915	1.047300	6.79
3.4	1.01330	1.011510	1.90	12.4	1.04999	1.048140	6.90
3.6	1.01409	1.012298	2.02	12.6	1.05084	1.048980	7.02
3.8	1.01488	1.013089	2.13	12.8	1.05168	1.049822	7.13
4.0	1.01567	1.013881	2.24	13.0	1.05252	1.050665	7.24
4.2	1.01647	1.014673	2.35	13.2	1.05337	1.051510	7.35
4.4	1.01726	1.015467	2.46	13.4	1.05422	1.052356	7.46
4.6	1.01806	1.016261	2.57	13.6	1.05506	1.053202	7.57
4.8	1.01886	1.017058	2.68	13.8	1.05591	1.054050	7.68
5.0	1.01965	1.017854	2.79	14.0	1.05677	1.054900	7.79
5.2	1.02045	1.018652	2.91	14.2	1.05762	1.055751	7.90
5.4	1.02125	1.019451	3.02	14.4	1.05847	1.056602	8.01
5.6	1.02206	1.020251	3.13	14.6	1.05933	1.057455	8.12
5.8	1.02286	1.021053	3.24	14.8	1.06018	1.058310	8.23
6.0	1.02366	1.021855	3.35	15.0	1.06104	1.059165	8.34
6.2	1.02447	1.022659	3.46	15.2	1.06190	1.060022	8.45
6.4	1.02527	1.023463	3.57	15.4	1.06276	1.060880	8.56
6.6	1.02608	1.024270	3.69	15.6	1.06362	1.061738	8.67
6.8	1.02689	1.025077	3.80	15.8	1.06448	1.062598	8.78
7.0	1.02770	1.025885	3.91	16.0	1.06534	1.063460	8.89
7.2	1.02851	1.026694	4.02	16.2	1.06621	1.064324	9.00
7.4	1.02932	1.027504	4.13	16.4	1.06707	1.065188	9.11
7.6	1.03013	1.028316	4.24	16.6	1.06794	1.066054	9.22
7.8	1.03095	1.029128	4.35	16.8	1.06881	1.066921	9.33
8.0	1.03176	1.029942	4.46	17.0	1.06968	1.067789	9.45
8.2	1.03258	1.030757	4.58	17.2	1.07055	1.068658	9.56
8.4	1.03340	1.031573	4.69	17.4	1.07142	1.069529	9.67
8.6	1.03422	1.032391	4.80	17.6	1.07229	1.070400	9.78
8.8	1.03504	1.033209	4.91	17.8	1.07317	1.071273	9.89

<sup>1</sup> Bur. Standards Circ. 44, 1918, p. 151.<sup>2</sup> Based upon figures prepared by the Kaiserliche Normal-Eichungs-Kommission and accepted by the International Commission for Unifying Methods of Sugar Analysis.

## 3 Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
18.0	1.07404	1.072147	10.00	27.0	1.11480	1.112828	14.93
18.2	1.07492	1.073023	10.11	27.2	1.11573	1.113763	15.04
18.4	1.07580	1.073900	10.22	27.4	1.11667	1.114697	15.15
18.6	1.07668	1.074777	10.33	27.6	1.11761	1.115635	15.26
18.8	1.07756	1.075657	10.44	27.8	1.11855	1.116572	15.37
19.0	1.07844	1.076537	10.55	28.0	1.11949	1.117512	15.48
19.2	1.07932	1.077419	10.66	28.2	1.12043	1.118453	15.59
19.4	1.08021	1.078302	10.77	28.4	1.12138	1.119395	15.69
19.6	1.08110	1.079187	10.88	28.6	1.12232	1.120339	15.80
19.8	1.08198	1.080072	10.99	28.8	1.12327	1.121284	15.91
20.0	1.08287	1.080959	11.10	29.0	1.12422	1.122231	16.02
20.2	1.08376	1.081848	11.21	29.2	1.12517	1.123179	16.13
20.4	1.08465	1.082737	11.32	29.4	1.12612	1.124128	16.24
20.6	1.08554	1.083628	11.43	29.6	1.12707	1.125079	16.35
20.8	1.08644	1.084520	11.54	29.8	1.12802	1.126030	16.46
21.0	1.08733	1.085414	11.65	30.0	1.12898	1.126984	16.57
21.2	1.08823	1.086309	11.76	30.2	1.12993	1.127939	16.67
21.4	1.08913	1.087205	11.87	30.4	1.13089	1.128896	16.78
21.6	1.09003	1.088101	11.98	30.6	1.13185	1.129853	16.89
21.8	1.09093	1.089000	12.09	30.8	1.13281	1.130812	17.00
22.0	1.09183	1.089900	12.20	31.0	1.13378	1.131773	17.11
22.2	1.09273	1.090802	12.31	31.2	1.13474	1.132735	17.22
22.4	1.09364	1.091704	12.42	31.4	1.13570	1.133698	17.33
22.6	1.09454	1.092607	12.52	31.6	1.13667	1.134663	17.43
22.8	1.09545	1.093513	12.63	31.8	1.13764	1.135628	17.54
23.0	1.09636	1.094420	12.74	32.0	1.13861	1.136596	17.65
23.2	1.09727	1.095328	12.85	32.2	1.13958	1.137565	17.76
23.4	1.09818	1.096236	12.96	32.4	1.14055	1.138534	17.87
23.6	1.09909	1.097147	13.07	32.6	1.14152	1.139506	17.98
23.8	1.10000	1.098058	13.18	32.8	1.14250	1.140479	18.08
24.0	1.10092	1.098971	13.29	33.0	1.14347	1.141453	18.19
24.2	1.10183	1.099886	13.40	33.2	1.14445	1.142429	18.30
24.4	1.10275	1.100802	13.51	33.4	1.14543	1.143405	18.41
24.6	1.10367	1.101718	13.62	33.6	1.14641	1.144384	18.52
24.8	1.10459	1.102637	13.73	33.8	1.14739	1.145363	18.63
25.0	1.10551	1.103557	13.84	34.0	1.14837	1.146345	18.73
25.2	1.10643	1.104478	13.95	34.2	1.14936	1.147328	18.84
25.4	1.10736	1.105400	14.06	34.4	1.15034	1.148313	18.95
25.6	1.10828	1.106324	14.17	34.6	1.15133	1.149298	19.06
25.8	1.10921	1.107248	14.28	34.8	1.15232	1.150286	19.17
26.0	1.11014	1.108175	14.39	35.0	1.15331	1.151275	19.28
26.2	1.11106	1.109103	14.49	35.2	1.15430	1.152265	19.38
26.4	1.11200	1.110033	14.60	35.4	1.15530	1.153256	19.49
26.6	1.11293	1.110963	14.71	35.6	1.15629	1.154249	19.60
26.8	1.11386	1.111895	14.82	35.8	1.15729	1.155242	19.71

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued. 3*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
36.0	1.15828	1.156238	19.81	45.0	1.20467	1.202540	24.63
36.2	1.15928	1.157235	19.92	45.2	1.20573	1.203603	24.74
36.4	1.16028	1.158233	20.03	45.4	1.20680	1.204668	24.85
36.6	1.16128	1.159233	20.14	45.6	1.20787	1.205733	24.95
36.8	1.16228	1.160233	20.25	45.8	1.20894	1.206801	25.06
37.0	1.16329	1.161236	20.35	46.0	1.21001	1.207870	25.17
37.2	1.16430	1.162240	20.46	46.2	1.21108	1.208940	25.27
37.4	1.16530	1.163245	20.57	46.4	1.21215	1.210013	25.38
37.6	1.16631	1.164252	20.68	46.6	1.21323	1.211086	25.48
37.8	1.16732	1.165259	20.78	46.8	1.21431	1.212162	25.59
38.0	1.16833	1.166269	20.89	47.0	1.21538	1.213238	25.70
38.2	1.16934	1.167281	21.00	47.2	1.21646	1.214317	25.80
38.4	1.17036	1.168293	21.11	47.4	1.21755	1.215395	25.91
38.6	1.17138	1.169307	21.21	47.6	1.21863	1.216476	26.01
38.8	1.17239	1.170322	21.32	47.8	1.21971	1.217559	26.12
39.0	1.17341	1.171340	21.43	48.0	1.22080	1.218643	26.23
39.2	1.17443	1.172359	21.54	48.2	1.22189	1.219729	26.33
39.4	1.17545	1.173379	21.64	48.4	1.22298	1.220815	26.44
39.6	1.17648	1.174400	21.75	48.6	1.22406	1.221904	26.54
39.8	1.17750	1.175423	21.86	48.8	1.22516	1.222995	26.65
40.0	1.17853	1.176447	21.97	49.0	1.22625	1.224086	26.75
40.2	1.17956	1.177473	22.07	49.2	1.22735	1.225180	26.86
40.4	1.18058	1.178501	22.18	49.4	1.22844	1.226274	26.96
40.6	1.18162	1.179527	22.29	49.6	1.22954	1.227371	27.07
40.8	1.18265	1.180560	22.39	49.8	1.23064	1.228469	27.18
41.0	1.18368	1.181592	22.50	50.0	1.23174	1.229567	27.28
41.2	1.18472	1.182625	22.61	50.2	1.23284	1.230668	27.39
41.4	1.18575	1.183660	22.72	50.4	1.23395	1.231770	27.49
41.6	1.18679	1.184696	22.82	50.6	1.23506	1.232874	27.60
41.8	1.18783	1.185734	22.93	50.8	1.23616	1.233979	27.70
42.0	1.18887	1.186773	23.04	51.0	1.23727	1.235085	27.81
42.2	1.18992	1.187814	23.14	51.2	1.23838	1.236194	27.91
42.4	1.19096	1.188856	23.25	51.4	1.23949	1.237303	28.02
42.6	1.19201	1.189901	23.36	51.6	1.24060	1.238414	28.12
42.8	1.19305	1.190946	23.46	51.8	1.24172	1.239527	28.23
43.0	1.19410	1.191993	23.57	52.0	1.24284	1.240641	28.33
43.2	1.19515	1.193041	23.68	52.2	1.24395	1.241757	28.44
43.4	1.19620	1.194090	23.78	52.4	1.24507	1.242873	28.54
43.6	1.19726	1.195141	23.89	52.6	1.24619	1.243992	28.65
43.8	1.19831	1.196193	24.00	52.8	1.24731	1.245113	28.75
44.0	1.19936	1.197247	24.10	53.0	1.24844	1.246234	28.86
44.2	1.20042	1.198303	24.21	53.2	1.24956	1.247358	28.96
44.4	1.20148	1.199360	24.32	53.4	1.25069	1.248482	29.06
44.6	1.20254	1.200420	24.42	53.6	1.25182	1.249609	29.17
44.8	1.20360	1.201480	24.53	53.8	1.25295	1.250737	29.27

## 3 Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
54.0	1.25408	1.251866	29.38	63.0	1.30657	1.304267	34.02
54.2	1.25521	1.252997	29.48	63.2	1.30778	1.305467	34.12
54.4	1.25635	1.254129	29.59	63.4	1.30898	1.306669	34.23
54.6	1.25748	1.255264	29.69	63.6	1.31019	1.307872	34.33
54.8	1.25862	1.256400	29.80	63.8	1.31139	1.309077	34.43
55.0	1.25976	1.257535	29.90	64.0	1.31260	1.310282	34.53
55.2	1.26090	1.258674	30.00	64.2	1.31381	1.311489	34.63
55.4	1.26204	1.259815	30.11	64.4	1.31502	1.312699	34.74
55.6	1.26319	1.260955	30.21	64.6	1.31623	1.313909	34.84
55.8	1.26433	1.262099	30.32	64.8	1.31745	1.315121	34.94
56.0	1.26548	1.263243	30.42	65.0	1.31866	1.316334	35.04
56.2	1.26663	1.264390	30.52	65.2	1.31988	1.317549	35.14
56.4	1.26778	1.265537	30.63	65.4	1.32110	1.318766	35.24
56.6	1.26893	1.266686	30.73	65.6	1.32232	1.319983	35.34
56.8	1.27008	1.267837	30.83	65.8	1.32354	1.321203	35.45
57.0	1.27123	1.268989	30.94	66.0	1.32476	1.322425	35.55
57.2	1.27239	1.270143	31.04	66.2	1.32599	1.323648	35.65
57.4	1.27355	1.271299	31.15	66.4	1.32722	1.324872	35.75
57.6	1.27471	1.272455	31.25	66.6	1.32844	1.326097	35.85
57.8	1.27587	1.273614	31.35	66.8	1.32967	1.327325	35.95
58.0	1.27703	1.274774	31.46	67.0	1.33090	1.328554	36.05
58.2	1.27819	1.275936	31.56	67.2	1.33214	1.329785	36.15
58.4	1.27936	1.277098	31.66	67.4	1.33337	1.331017	36.25
58.6	1.28052	1.278262	31.76	67.6	1.33460	1.332250	36.35
58.8	1.28169	1.279428	31.87	67.8	1.33584	1.333485	36.45
59.0	1.28286	1.280595	31.97	68.0	1.33708	1.334722	36.55
59.2	1.28404	1.281764	32.07	68.2	1.33832	1.335961	36.66
59.4	1.28520	1.282935	32.18	68.4	1.33957	1.337200	36.76
59.6	1.28638	1.284107	32.28	68.6	1.34081	1.338441	36.86
59.8	1.28755	1.285281	32.38	68.8	1.34205	1.339684	36.96
60.0	1.28873	1.286456	32.49	69.0	1.34330	1.340928	37.06
60.2	1.28991	1.287633	32.59	69.2	1.34455	1.342174	37.16
60.4	1.29109	1.288811	32.69	69.4	1.34580	1.343421	37.26
60.6	1.29227	1.289991	32.79	69.6	1.34705	1.344671	37.36
60.8	1.29346	1.291172	32.90	69.8	1.34830	1.345922	37.46
61.0	1.29464	1.292354	33.00	70.0	1.34956	1.347174	37.56
61.2	1.29583	1.293539	33.10	70.2	1.35081	1.348427	37.66
61.4	1.29701	1.294725	33.20	70.4	1.35207	1.349682	37.76
61.6	1.29820	1.295911	33.31	70.6	1.35333	1.350939	37.86
61.8	1.29940	1.297100	33.41	70.8	1.35459	1.352197	37.96
62.0	1.30059	1.298291	33.51	71.0	1.35585	1.353456	38.06
62.2	1.30178	1.299483	33.61	71.2	1.35711	1.354717	38.16
62.4	1.30298	1.300677	33.72	71.4	1.35838	1.355980	38.26
62.6	1.30418	1.301871	33.82	71.6	1.35964	1.357245	38.35
62.8	1.30537	1.303068	33.92	71.8	1.36091	1.358511	38.45



*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued. 3*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
72.0	1.36218	1.359778	38.55	81.0	1.42088	1.418374	42.95
72.2	1.36346	1.361047	38.65	81.2	1.42222	1.419711	43.05
72.4	1.36473	1.362317	38.75	81.4	1.42356	1.421049	43.14
72.6	1.36600	1.363590	38.85	81.6	1.42490	1.422390	43.24
72.8	1.36728	1.364864	38.95	81.8	1.42625	1.423730	43.33
73.0	1.36856	1.366139	39.05	82.0	1.42759	1.425072	43.43
73.2	1.36983	1.367415	39.15	82.2	1.42894	1.426416	43.53
73.4	1.37111	1.368693	39.25	82.4	1.43029	1.427761	43.62
73.6	1.37240	1.369973	39.35	82.6	1.43164	1.429109	43.72
73.8	1.37368	1.371254	39.44	82.8	1.43298	1.430457	43.81
74.0	1.37496	1.372536	39.54	83.0	1.43434	1.431807	43.91
74.2	1.37625	1.373820	39.64	83.2	1.43569	1.433158	44.00
74.4	1.37754	1.375105	39.74	83.4	1.43705	1.434511	44.10
74.6	1.37883	1.376392	39.84	83.6	1.43841	1.435866	44.19
74.8	1.38012	1.377680	39.94	83.8	1.43976	1.437222	44.29
75.0	1.38141	1.378971	40.03	84.0	1.44112	1.438579	44.38
75.2	1.38270	1.380262	40.13	84.2	1.44249	1.439938	44.48
75.4	1.38400	1.381555	40.23	84.4	1.44385	1.441299	44.57
75.6	1.38530	1.382851	40.33	84.6	1.44521	1.442661	44.67
75.8	1.38660	1.384148	40.43	84.8	1.44658	1.444024	44.76
76.0	1.38790	1.385446	40.53	85.0	1.44794	1.445388	44.86
76.2	1.38920	1.386745	40.62	85.2	1.44931	1.446754	44.95
76.4	1.39050	1.388045	40.72	85.4	1.45068	1.448121	45.05
76.6	1.39180	1.389347	40.82	85.6	1.45205	1.449491	45.14
76.8	1.39311	1.390651	40.92	85.8	1.45343	1.450860	45.24
77.0	1.39442	1.391956	41.01	86.0	1.45480	1.452232	45.33
77.2	1.39573	1.393263	41.11	86.2	1.45618	1.453605	45.42
77.4	1.39704	1.394571	41.21	86.4	1.45755	1.454980	45.52
77.6	1.39835	1.395881	41.31	86.6	1.45893	1.456357	45.61
77.8	1.39966	1.397192	41.40	86.8	1.46031	1.457735	45.71
78.0	1.40098	1.398505	41.50	87.0	1.46170	1.459114	45.80
78.2	1.40230	1.399819	41.60	87.2	1.46308	1.460495	45.89
78.4	1.40361	1.401134	41.70	87.4	1.46446	1.461877	45.99
78.6	1.40493	1.402452	41.79	87.6	1.46585	1.463260	46.08
78.8	1.40625	1.403771	41.89	87.8	1.46724	1.464645	46.17
79.0	1.40758	1.405091	41.99	88.0	1.46862	1.466032	46.27
79.2	1.40890	1.406412	42.08	88.2	1.47002	1.467420	46.36
79.4	1.41023	1.407735	42.18	88.4	1.47141	1.468810	46.45
79.6	1.41155	1.409061	42.28	88.6	1.47280	1.470200	46.55
79.8	1.41288	1.410387	42.37	88.8	1.47420	1.471592	46.64
80.0	1.41421	1.411715	42.47	89.0	1.47559	1.472986	46.73
80.2	1.41554	1.413044	42.57	89.2	1.47699	1.474381	46.83
80.4	1.41688	1.414374	42.66	89.4	1.47839	1.475779	46.92
80.6	1.41821	1.415706	42.76	89.6	1.47979	1.477176	47.01
80.8	1.41955	1.417039	42.85	89.8	1.48119	1.478575	47.11

## 3 Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Concluded.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
90.0	1.48259	1.479976	47.20	95.0	1.51814	1.515455	49.49
90.2	1.48400	1.481378	47.29	95.2	1.51958	1.516893	49.58
90.4	1.48540	1.482782	47.38	95.4	1.52102	1.518332	49.67
90.6	1.48681	1.484187	47.48	95.6	1.52246	1.519771	49.76
90.8	1.48822	1.485593	47.57	95.8	1.52390	1.521212	49.85
91.0	1.48963	1.487002	47.66	96.0	1.52535	1.522656	49.94
91.2	1.49104	1.488411	47.75	96.2	1.52680	1.524100	50.03
91.4	1.49246	1.489823	47.84	96.4	1.52824	1.525546	50.12
91.6	1.49387	1.491234	47.94	96.6	1.52969	1.526993	50.21
91.8	1.49529	1.492647	48.03	96.8	1.53114	1.528441	50.30
92.0	1.49671	1.494063	48.12	97.0	1.53260	1.529891	50.39
92.2	1.49812	1.495479	48.21	97.2	1.53405	1.531342	50.48
92.4	1.49954	1.496897	48.30	97.4	1.53551	1.532794	50.57
92.6	1.50097	1.498316	48.40	97.6	1.53696	1.534248	50.66
92.8	1.50239	1.499736	48.49	97.8	1.53842	1.535704	50.75
93.0	1.50381	1.501158	48.58	98.0	1.53988	1.537161	50.84
93.2	1.50524	1.502582	48.67	98.2	1.54134	1.538618	50.93
93.4	1.50667	1.504006	48.76	98.4	1.54280	1.540076	51.02
93.6	1.50810	1.505432	48.85	98.6	1.54426	1.541536	51.10
93.8	1.50952	1.506859	48.94	98.8	1.54573	1.542998	51.19
94.0	1.51096	1.508289	49.03	99.0	1.54719	1.544462	51.28
94.2	1.51239	1.509720	49.12	99.2	1.54866	1.545926	51.37
94.4	1.51382	1.511151	49.22	99.4	1.55013	1.547392	51.46
94.6	1.51526	1.512585	49.31	99.6	1.55160	1.548861	51.55
94.8	1.51670	1.514019	49.40	99.8	1.55307	1.550329	51.64
				100.0	1.55454	1.551800	51.73

*Temperature corrections to readings of saccharimeters (standard at 20°C). 4*

(This table is calculated from the data on thermal expansion of sugar solutions by Plato,<sup>1</sup> and it is assumed that the instrument is of Jena 16<sup>mm</sup> glass. The table should be used with caution and only for approximate results when the temperature differs much from the standard temperature or from the temperature of the surrounding air.)

TEMPERATURE IN DEGREES CENTIGRADE	OBSERVED PERCENTAGE OF SUGAR													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract—													
0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.49
5	0.36	0.47	0.56	0.65	0.73	0.80	0.86	0.91	0.97	1.01	1.05	1.08	1.10	1.14
10	0.32	0.38	0.43	0.48	0.52	0.57	0.60	0.64	0.67	0.70	0.72	0.74	0.75	0.77
11	0.31	0.35	0.40	0.44	0.48	0.51	0.55	0.58	0.60	0.63	0.65	0.66	0.68	0.70
12	0.29	0.32	0.36	0.40	0.43	0.46	0.50	0.52	0.54	0.56	0.58	0.59	0.60	0.62
13	0.26	0.29	0.32	0.35	0.38	0.41	0.44	0.46	0.48	0.49	0.51	0.52	0.53	0.55
14	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47
15	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.33	0.34	0.36	0.36	0.37	0.38	0.39
16	0.17	0.18	0.20	0.22	0.23	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32
17	0.13	0.14	0.15	0.16	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24
18	0.09	0.10	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16
19	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
17.5	0.11	0.12	0.12	0.14	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20
15.56 (60°F)	0.18	0.20	0.22	0.24	0.26	0.28	0.29	0.30	0.30	0.32	0.33	0.33	0.34	0.34
	Add—													
21	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09
22	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.16
23	0.16	0.16	0.17	0.17	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.24	0.24	0.24
24	0.21	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.32	0.32
25	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.38	0.38	0.39	0.39	0.40	0.39
26	0.33	0.34	0.36	0.37	0.40	0.40	0.42	0.44	0.46	0.47	0.47	0.48	0.48	0.48
27	0.40	0.41	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.54	0.55	0.56	0.56	0.56
28	0.46	0.47	0.49	0.51	0.54	0.56	0.58	0.60	0.61	0.62	0.63	0.64	0.64	0.64
29	0.54	0.55	0.56	0.59	0.61	0.63	0.66	0.68	0.70	0.70	0.71	0.72	0.72	0.72
30	0.61	0.62	0.63	0.66	0.68	0.71	0.73	0.76	0.78	0.78	0.79	0.80	0.80	0.81
35	0.99	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22
40	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65
45	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08
50	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52
55	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97
60	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43
27.5	0.43	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.58	0.59	0.60	0.60	0.60

<sup>1</sup> Wiss. Abh. Kaiserliche Normal-Eichungs-Kommission, Vol. 2, 1900, p. 140.

5 *Domke's table of apparent specific gravity of sucrose solutions at 20°C.<sup>1</sup>*

Calculated from the tables of the Kaiserliche Normal-Eichungs-Kommission and accepted by the International Commission for Unifying Methods of Sugar Analysis.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	1.0000	1.0004	1.0008	1.0012	1.0016	1.0019	1.0023	1.0027	1.0031	1.0035
1	1.0039	1.0043	1.0047	1.0051	1.0055	1.0058	1.0062	1.0066	1.0070	1.0074
2	1.0078	1.0082	1.0086	1.0090	1.0094	1.0098	1.0102	1.0106	1.0109	1.0013
3	1.0117	1.0121	1.0125	1.0129	1.0133	1.0137	1.0141	1.0145	1.0149	1.0153
4	1.0157	1.0161	1.0165	1.0169	1.0173	1.0177	1.0181	1.0185	1.0189	1.0193
5	1.0197	1.0201	1.0205	1.0209	1.0213	1.0217	1.0221	1.0225	1.0229	1.0233
6	1.0237	1.0241	1.0245	1.0249	1.0253	1.0257	1.0261	1.0265	1.0269	1.0273
7	1.0277	1.0281	1.0285	1.0289	1.0294	1.0298	1.0302	1.0306	1.0310	1.0314
8	1.0318	1.0322	1.0326	1.0330	1.0334	1.0338	1.0343	1.0347	1.0351	1.0355
9	1.0359	1.0363	1.0367	1.0371	1.0375	1.0380	1.0384	1.0388	1.0392	1.0396
10	1.0400	1.0404	1.0409	1.0413	1.0417	1.0421	1.0425	1.0429	1.0433	1.0438
11	1.0442	1.0446	1.0450	1.0454	1.0459	1.0463	1.0467	1.0471	1.0475	1.0480
12	1.0484	1.0488	1.0492	1.0496	1.0501	1.0505	1.0509	1.0513	1.0517	1.0522
13	1.0526	1.0530	1.0534	1.0539	1.0543	1.0547	1.0551	1.0556	1.0560	1.0564
14	1.0568	1.0573	1.0577	1.0581	1.0585	1.0589	1.0594	1.0598	1.0603	1.0607
15	1.0611	1.0615	1.0620	1.0624	1.0628	1.0633	1.0637	1.0641	1.0646	1.0650
16	1.0654	1.0659	1.0663	1.0667	1.0672	1.0676	1.0680	1.0685	1.0689	1.0693
17	1.0698	1.0702	1.0706	1.0711	1.0715	1.0719	1.0724	1.0728	1.0733	1.0737
18	1.0741	1.0746	1.0750	1.0755	1.0759	1.0763	1.0768	1.0772	1.0777	1.0781
19	1.0785	1.0790	1.0794	1.0799	1.0803	1.0807	1.0812	1.0816	1.0821	1.0825
20	1.0830	1.0834	1.0839	1.0843	1.0848	1.0852	1.0856	1.0861	1.0865	1.0870
21	1.0874	1.0879	1.0883	1.0888	1.0892	1.0897	1.0901	1.0905	1.0910	1.0915
22	1.0919	1.0924	1.0928	1.0933	1.0937	1.0942	1.0946	1.0951	1.0956	1.0960
23	1.0965	1.0969	1.0974	1.0978	1.0983	1.0987	1.0992	1.0997	1.1001	1.1006
24	1.1010	1.1015	1.1020	1.1024	1.1029	1.1033	1.1038	1.1043	1.1047	1.1052
25	1.1056	1.1061	1.1066	1.1070	1.1075	1.1079	1.1084	1.1089	1.1093	1.1098
26	1.1103	1.1107	1.1112	1.1117	1.1121	1.1126	1.1131	1.1135	1.1140	1.1145
27	1.1149	1.1154	1.1159	1.1163	1.1168	1.1173	1.1178	1.1182	1.1187	1.1192
28	1.1196	1.1201	1.1206	1.1210	1.1215	1.1220	1.1225	1.1229	1.1234	1.1239
29	1.1244	1.1248	1.1253	1.1258	1.1263	1.1267	1.1272	1.1277	1.1282	1.1287
30	1.1291	1.1296	1.1301	1.1306	1.1311	1.1315	1.1320	1.1325	1.1330	1.1334
31	1.1339	1.1344	1.1349	1.1354	1.1359	1.1363	1.1368	1.1373	1.1378	1.1383
32	1.1388	1.1393	1.1397	1.1402	1.1407	1.1412	1.1417	1.1422	1.1427	1.1432
33	1.1436	1.1441	1.1446	1.1451	1.1456	1.1461	1.1466	1.1471	1.1476	1.1481
34	1.1486	1.1490	1.1495	1.1500	1.1505	1.1510	1.1515	1.1520	1.1525	1.1530
35	1.1535	1.1540	1.1545	1.1550	1.1555	1.1560	1.1565	1.1570	1.1575	1.1580
36	1.1585	1.1590	1.1595	1.1600	1.1605	1.1610	1.1615	1.1620	1.1625	1.1630
37	1.1635	1.1640	1.1645	1.1650	1.1655	1.1660	1.1665	1.1670	1.1675	1.1680
38	1.1685	1.1690	1.1696	1.1701	1.1706	1.1711	1.1716	1.1721	1.1726	1.1731
39	1.1736	1.1741	1.1746	1.1752	1.1757	1.1762	1.1767	1.1772	1.1777	1.1782
40	1.1787	1.1793	1.1798	1.1803	1.1808	1.1813	1.1818	1.1824	1.1829	1.1834
41	1.1839	1.1844	1.1849	1.1855	1.1860	1.1865	1.1870	1.1875	1.1881	1.1886
42	1.1891	1.1896	1.1901	1.1907	1.1912	1.1917	1.1922	1.1928	1.1933	1.1938
43	1.1943	1.1949	1.1954	1.1959	1.1964	1.1970	1.1975	1.1980	1.1985	1.1991
44	1.1996	1.2001	1.2007	1.2012	1.2017	1.2023	1.2028	1.2033	1.2039	1.2044

<sup>1</sup> Z. Ver. deut. Zucker-Ind., 62, 306 (1912).

*Domke's table of apparent specific gravity of sucrose solutions at 20°C.—Concluded. 5*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
45	1.2049	1.2054	1.2060	1.2065	1.2070	1.2076	1.2081	1.2087	1.2092	1.2097
46	1.2102	1.2108	1.2113	1.2118	1.2124	1.2129	1.2135	1.2140	1.2146	1.2151
47	1.2156	1.2162	1.2167	1.2173	1.2178	1.2184	1.2189	1.2194	1.2200	1.2205
48	1.2211	1.2216	1.2222	1.2227	1.2232	1.2238	1.2243	1.2249	1.2254	1.2260
49	1.2265	1.2271	1.2276	1.2282	1.2287	1.2293	1.2298	1.2304	1.2309	1.2315
50	1.2320	1.2326	1.2331	1.2337	1.2342	1.2348	1.2353	1.2359	1.2364	1.2370
51	1.2376	1.2381	1.2387	1.2392	1.2398	1.2403	1.2409	1.2415	1.2420	1.2426
52	1.2431	1.2437	1.2442	1.2448	1.2454	1.2459	1.2465	1.2471	1.2476	1.2482
53	1.2487	1.2493	1.2499	1.2504	1.2510	1.2516	1.2521	1.2527	1.2533	1.2538
54	1.2544	1.2550	1.2555	1.2561	1.2567	1.2572	1.2578	1.2584	1.2589	1.2595
55	1.2601	1.2606	1.2612	1.2618	1.2624	1.2629	1.2635	1.2641	1.2647	1.2652
56	1.2658	1.2664	1.2670	1.2675	1.2681	1.2687	1.2693	1.2698	1.2704	1.2710
57	1.2716	1.2721	1.2727	1.2733	1.2739	1.2745	1.2750	1.2756	1.2762	1.2768
58	1.2774	1.2779	1.2785	1.2791	1.2797	1.2803	1.2809	1.2815	1.2821	1.2826
59	1.2832	1.2838	1.2844	1.2850	1.2856	1.2861	1.2867	1.2873	1.2879	1.2885
60	1.2891	1.2897	1.2903	1.2909	1.2914	1.2920	1.2926	1.2932	1.2938	1.2944
61	1.2950	1.2956	1.2962	1.2968	1.2974	1.2980	1.2986	1.2992	1.2998	1.3004
62	1.3010	1.3015	1.3021	1.3027	1.3033	1.3039	1.3045	1.3051	1.3057	1.3063
63	1.3069	1.3075	1.3081	1.3087	1.3093	1.3100	1.3106	1.3112	1.3118	1.3124
64	1.3130	1.3136	1.3142	1.3148	1.3154	1.3160	1.3166	1.3172	1.3178	1.3184
65	1.3190	1.3197	1.3203	1.3209	1.3215	1.3221	1.3227	1.3233	1.3239	1.3245
66	1.3252	1.3258	1.3264	1.3270	1.3276	1.3282	1.3288	1.3295	1.3301	1.3307
67	1.3313	1.3319	1.3325	1.3332	1.3338	1.3344	1.3350	1.3356	1.3363	1.3369
68	1.3375	1.3381	1.3387	1.3394	1.3400	1.3406	1.3412	1.3418	1.3425	1.3431
69	1.3437	1.3443	1.3450	1.3456	1.3462	1.3468	1.3475	1.3481	1.3487	1.3494
70	1.3500	1.3506	1.3512	1.3519	1.3525	1.3531	1.3538	1.3544	1.3550	1.3557
71	1.3563	1.3569	1.3575	1.3582	1.3588	1.3594	1.3601	1.3607	1.3614	1.3620
72	1.3626	1.3633	1.3639	1.3645	1.3652	1.3658	1.3664	1.3671	1.3677	1.3684
73	1.3690	1.3696	1.3703	1.3709	1.3716	1.3722	1.3729	1.3735	1.3741	1.3748
74	1.3754	1.3761	1.3767	1.3774	1.3780	1.3786	1.3793	1.3799	1.3806	1.3812
75	1.3819	1.3825	1.3832	1.3838	1.3845	1.3851	1.3858	1.3864	1.3871	1.3877
76	1.3884	1.3890	1.3897	1.3903	1.3910	1.3916	1.3923	1.3929	1.3936	1.3942
77	1.3949	1.3955	1.3962	1.3969	1.3975	1.3982	1.3988	1.3995	1.4001	1.4008
78	1.4015	1.4021	1.4028	1.4034	1.4041	1.4048	1.4054	1.4061	1.4067	1.4074
79	1.4081	1.4087	1.4094	1.4101	1.4107	1.4114	1.4121	1.4127	1.4134	1.4140
80	1.4147	1.4154	1.4160	1.4167	1.4174	1.4180	1.4187	1.4194	1.4201	1.4207
81	1.4214	1.4221	1.4227	1.4234	1.4241	1.4247	1.4254	1.4261	1.4268	1.4274
82	1.4281	1.4288	1.4295	1.4301	1.4308	1.4315	1.4322	1.4328	1.4335	1.4342
83	1.4349	1.4355	1.4362	1.4369	1.4376	1.4383	1.4389	1.4396	1.4403	1.4410
84	1.4417	1.4423	1.4430	1.4437	1.4444	1.4451	1.4458	1.4464	1.4471	1.4478
85	1.4485	1.4492	1.4499	1.4505	1.4512	1.4519	1.4526	1.4533	1.4540	1.4547
86	1.4554	1.4560	1.4567	1.4574	1.4581	1.4588	1.4595	1.4602	1.4609	1.4616
87	1.4623	1.4629	1.4636	1.4643	1.4650	1.4657	1.4664	1.4671	1.4678	1.4685
88	1.4692	1.4699	1.4706	1.4713	1.4720	1.4727	1.4734	1.4741	1.4748	1.4755
89	1.4762	1.4769	1.4776	1.4783	1.4790	1.4797	1.4804	1.4811	1.4818	1.4825
90	1.4832									

6

*Refractive indices of sucrose solutions at 20°C.<sup>1</sup>*  
(International Scale, 1936)<sup>2</sup>

REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT
1.33299	0.0	1.34629	9.0	1.36053	18.0	1.3758	27.0	1.3920	36.0
.33328	0.2	.34660	9.2	.36086	18.2	.3761	27.2	.3924	36.2
.33357	0.4	.34691	9.4	.36119	18.4	.3765	27.4	.3928	36.4
.33385	0.6	.34721	9.6	.36152	18.6	.3768	27.6	.3931	36.6
.33414	0.8	.34752	9.8	.36185	18.8	.3772	27.8	.3935	36.8
.33443	1.0	.34783	10.0	.36218	19.0	.3775	28.0	.3939	37.0
.33472	1.2	.34814	10.2	.36251	19.2	.3779	28.2	.3943	37.2
.33501	1.4	.34845	10.4	.36284	19.4	.3782	28.4	.3947	37.4
.33530	1.6	.34875	10.6	.36318	19.6	.3786	28.6	.3950	37.6
.33559	1.8	.34906	10.8	.36351	19.8	.3789	28.8	.3954	37.8
.33588	2.0	.34937	11.0	.36384	20.0	.3793	29.0	.3958	38.0
.33617	2.2	.34968	11.2	.36417	20.2	.3797	29.2	.3962	38.2
.33646	2.4	.34999	11.4	.36451	20.4	.3800	29.4	.3966	38.4
.33675	2.6	.35031	11.6	.36484	20.6	.3804	29.6	.3970	38.6
.33704	2.8	.35062	11.8	.36518	20.8	.3807	29.8	.3974	38.8
.33733	3.0	.35093	12.0	.36551	21.0	.3811	30.0	.3978	39.0
.33762	3.2	.35124	12.2	.36585	21.2	.3815	30.2	.3982	39.2
.33792	3.4	.35156	12.4	.36618	21.4	.3818	30.4	.3986	39.4
.33821	3.6	.35187	12.6	.36652	21.6	.3822	30.6	.3989	39.6
.33851	3.8	.35219	12.8	.36685	21.8	.3825	30.8	.3993	39.8
.33880	4.0	.35250	13.0	.36719	22.0	.3829	31.0	.3997	40.0
.33909	4.2	.35282	13.2	.36753	22.2	.3833	31.2	.4001	40.2
.33939	4.4	.35313	13.4	.36787	22.4	.3836	31.4	.4005	40.4
.33968	4.6	.35345	13.6	.36820	22.6	.3840	31.6	.4008	40.6
.33998	4.8	.35376	13.8	.36854	22.8	.3843	31.8	.4012	40.8
.34027	5.0	.35408	14.0	.36888	23.0	.3847	32.0	.4016	41.0
.34057	5.2	.35440	14.2	.36922	23.2	.3851	32.2	.4020	41.2
.34087	5.4	.35472	14.4	.36956	23.4	.3854	32.4	.4024	41.4
.34116	5.6	.35503	14.6	.36991	23.6	.3858	32.6	.4028	41.6
.34146	5.8	.35535	14.8	.37025	23.8	.3861	32.8	.4032	41.8
.34176	6.0	.35567	15.0	.37059	24.0	.3865	33.0	.4036	42.0
.34206	6.2	.35599	15.2	.3709	24.2	.3869	33.2	.4040	42.2
.34236	6.4	.35631	15.4	.3713	24.4	.3872	33.4	.4044	42.4
.34266	6.6	.35664	15.6	.3716	24.6	.3876	33.6	.4048	42.6
.34296	6.8	.35696	15.8	.3720	24.8	.3879	33.8	.4052	42.8
.34326	7.0	.35728	16.0	.3723	25.0	.3883	34.0	.4056	43.0
.34356	7.2	.35760	16.2	.3726	25.2	.3887	34.2	.4060	43.2
.34386	7.4	.35793	16.4	.3730	25.4	.3891	34.4	.4064	43.4
.34417	7.6	.35825	16.6	.3733	25.6	.3894	34.6	.4068	43.6
.34447	7.8	.35858	16.8	.3737	25.8	.3898	34.8	.4072	43.8
.34477	8.0	.35890	17.0	.3740	26.0	.3902	35.0	.4076	44.0
.34507	8.2	.35923	17.2	.3744	26.2	.3906	35.2	.4080	44.2
.34538	8.4	.35955	17.4	.3747	26.4	.3909	35.4	.4084	44.4
.34568	8.6	.35988	17.6	.3751	26.6	.3913	35.6	.4088	44.6
.34599	8.8	.36020	17.8	.3754	26.8	.3916	35.8	.4092	44.8

<sup>1</sup> This table is in accordance with the International Scale of Refractive Indices of Sucrose Solutions at 20° C, 1936, adopted as official (final action) at the 1938 meeting of the Association. The values of the indices for the range 0–24% sucrose are given to five decimal places instead of to four. This arrangement is desirable when the table is used with refractometers capable of readings to the fifth place. The values for whole per cents of sucrose are those of the International Scale; the remaining fractional values are obtained by interpolation. The values of indices above 24% sucrose are identical with those in Table 6, *Methods of Analysis*, A. O. A. C., 1935, p. 622.

<sup>2</sup> *Intern. Sugar J.*, 39, 22s (1937).



7 Table of corrections for determining percentage of sucrose in sugar solutions by means of either Abbé or immersion refractometer when readings are made at temperatures other than 20°C.<sup>1</sup>

(International Temperature Correction Table, 1936)<sup>1</sup>

TEMP. °C	PER CENT SUCROSE										
	0	5	10	15	20	25	30	40	50	60	70
	Subtract from the per cent sucrose										
10	0.50	0.54	0.58	0.61	0.64	0.66	0.68	0.72	0.74	0.76	0.79
11	.46	.49	.53	.55	.58	.60	.62	.65	.67	.69	.71
12	.42	.45	.48	.50	.52	.54	.56	.58	.60	.61	.63
13	.37	.40	.42	.44	.46	.48	.49	.51	.53	.54	.55
14	.33	.35	.37	.39	.40	.41	.42	.44	.45	.46	.48
15	.27	.29	.31	.33	.34	.34	.35	.37	.38	.39	.40
16	.22	.24	.25	.26	.27	.28	.28	.30	.30	.31	.32
17	.17	.18	.19	.20	.21	.21	.21	.22	.23	.23	.24
18	.12	.13	.13	.14	.14	.14	.14	.15	.15	.16	.16
19	.06	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08
	Add to the per cent sucrose										
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08
22	.13	.13	.14	.14	.15	.15	.15	.15	.16	.16	.16
23	.19	.20	.21	.22	.22	.23	.23	.23	.24	.24	.24
24	.26	.27	.28	.29	.30	.30	.31	.31	.31	.32	.32
25	.33	.35	.36	.37	.38	.38	.39	.40	.40	.40	.40
26	.40	.42	.43	.44	.45	.46	.47	.48	.48	.48	.48
27	.48	.50	.52	.53	.54	.55	.55	.56	.56	.56	.56
28	.56	.57	.60	.61	.62	.63	.63	.64	.64	.64	.64
29	.64	.66	.68	.69	.71	.72	.72	.73	.73	.73	.73
30	.72	.74	.77	.78	.79	.80	.80	.81	.81	.81	.81

<sup>1</sup> Intern. Sugar J., 39, 24s (1937).



Table for determining the percentage of sucrose in sugar solutions from the readings of the Zeiss immersion refractometer at 20°C.<sup>1</sup>

SCALE READING <sup>2</sup> 20°C.	$n_D^{20}$	SUCROSE PER CENT	SCALE READING 20°C.	$n_D^{20}$	SUCROSE PER CENT	SCALE READING 20°C.	$n_D^{20}$	SUCROSE PER CENT
14.47	1.33299	0	45	1.34463	7.91	76	1.35606	15.24
15	3320	0.15	46	4500	8.15	77	5642	15.47
16	3358	0.41	47	4537	8.39	78	5678	15.69
17	3397	0.68	48	4575	8.64	79	5714	15.91
18	3435	0.94	49	4612	8.89	80	5750	16.14
19	3474	1.21	50	4650	9.13	81	5786	16.36
20	3513	1.48	51	4687	9.38	82	5822	16.58
21	3551	1.74	52	4724	9.62	83	5858	16.81
22	3590	2.01	53	4761	9.86	84	5894	17.03
23	3628	2.27	54	4798	10.10	85	5930	17.25
24	3667	2.54	55	4836	10.34	86	5966	17.47
25	3705	2.80	56	4873	10.58	87	6002	17.69
26	3743	3.07	57	4910	10.82	88	6038	17.91
27	3781	3.33	58	4947	11.06	89	6074	18.12
28	3820	3.59	59	4984	11.30	90	6109	18.34
29	3858	3.85	60	5021	11.54	91	6145	18.56
30	3896	4.11	61	5058	11.78	92	6181	18.78
31	3934	4.36	62	5095	12.01	93	6217	19.00
32	3972	4.62	63	5132	12.25	94	6252	19.21
33	4010	4.88	64	5169	12.48	95	6287	19.42
34	4048	5.14	65	5205	12.72	96	6323	19.63
35	4086	5.40	66	5242	12.95	97	6359	19.85
36	4124	5.65	67	5279	13.18	98	6394	20.06
37	4162	5.91	68	5316	13.41	99	6429	20.27
38	4199	6.16	69	5352	13.64	100	6464	20.48
39	4237	6.41	70	5388	13.87	101	6500	20.69
40	4275	6.66	71	5425	14.10	102	6535	20.90
41	4313	6.91	72	5461	14.33	103	6570	21.11
42	4350	7.16	73	5497	14.56	104	6605	21.32
43	4388	7.41	74	5533	14.79	105	6640	21.53
44	4426	7.66	75	5569	15.01			

<sup>1</sup> The values in this table were calculated by J. A. Mathews from the five-place indices of Schönrock as given by Landt, *Z. Ver. deut. Zucker-Ind.*, 83, 692 (1933).

<sup>2</sup> The scale readings refer only to the scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168 (1899). According to this scale 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If the immersion refractometer used is calibrated according to another arbitrary scale, the readings must be converted into refractive indices before this table is used to determine the percentage of sugar.

9 *Munson and Walker's table for calculating dextrose, levulose, invert sugar alone, invert sugar in the presence of sucrose (0.4 gram and 2 grams total sugar), lactose, lactose and sucrose (2 mixtures), and maltose (crystallized).*<sup>1</sup>  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROROSE (d-GLUCOSE)	LEVULOSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
					0.4 gram total sugar	2 grams total sugar		1 lactose, 4 su- crose	1 lactose, 12 su- crose		
10	8.9	4.0	4.5	4.5	1.6	.....	6.3	6.1	.....	6.2	10
12	10.7	4.9	5.5	5.4	2.5	.....	7.5	7.3	.....	7.9	12
14	12.4	5.7	6.4	6.3	3.4	.....	8.8	8.5	.....	9.5	14
16	14.2	6.6	7.3	7.2	4.3	.....	10.0	9.7	.....	11.2	16
18	16.0	7.5	8.3	8.1	5.2	.....	11.3	10.9	.....	12.9	18
20	17.8	8.3	9.2	8.9	6.1	.....	12.5	12.1	.....	14.6	20
22	19.5	9.2	10.2	9.8	7.0	.....	13.8	13.3	.....	16.2	22
24	21.3	10.0	11.1	10.7	7.9	.....	15.0	14.5	.....	17.9	24
26	23.1	10.9	12.1	11.6	8.8	.....	16.3	15.8	.....	19.6	26
28	24.9	11.8	13.0	12.5	9.7	.....	17.6	17.0	.....	21.2	28
30	26.6	12.6	14.0	13.4	10.7	4.3	18.8	18.2	.....	22.9	30
32	28.4	13.5	14.9	14.3	11.6	5.2	20.1	19.4	.....	24.6	32
34	30.2	14.3	15.9	15.2	12.5	6.1	21.4	20.7	.....	26.2	34
36	32.0	15.2	16.8	16.1	13.4	7.0	22.8	22.0	.....	27.9	36
38	33.8	16.1	17.8	16.9	14.3	7.9	24.2	23.3	.....	29.6	38
40	35.5	16.9	18.7	17.8	15.2	8.8	25.5	24.7	.....	31.3	40
42	37.3	17.8	19.7	18.7	16.1	9.7	26.9	26.0	.....	32.9	42
44	39.1	18.7	20.6	19.6	17.0	10.7	28.3	27.3	.....	34.6	44
46	40.9	19.6	21.6	20.5	17.9	11.6	29.6	28.6	.....	36.3	46
48	42.6	20.4	22.6	21.4	18.8	12.5	31.0	30.0	.....	37.9	48
50	44.4	21.3	23.5	22.3	19.7	13.4	32.3	31.3	.....	39.6	50
52	46.2	22.2	24.5	23.2	20.7	14.3	33.7	32.6	.....	41.3	52
54	48.0	23.0	25.4	24.1	21.6	15.2	35.1	34.0	.....	42.9	54
56	49.7	23.9	26.4	25.0	22.5	16.2	36.4	35.3	.....	44.6	56
58	51.5	24.8	27.4	25.9	23.4	17.1	37.8	36.6	.....	46.3	58
60	53.3	25.6	28.3	26.8	24.3	18.0	39.2	37.9	.....	48.0	60
62	55.1	26.5	29.3	27.7	25.2	18.9	40.5	39.3	.....	49.6	62
64	56.8	27.4	30.2	28.6	26.2	19.8	41.9	40.6	.....	51.3	64
66	58.6	28.3	31.2	29.5	27.1	20.8	43.3	41.9	.....	53.0	66
68	60.4	29.2	32.2	30.4	28.0	21.7	44.7	43.3	40.7	54.6	68
70	62.2	30.0	33.1	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
72	64.0	30.9	34.1	32.3	29.8	23.5	47.4	45.9	43.1	58.0	72
74	65.7	31.8	35.1	33.2	30.8	24.5	48.8	47.3	44.2	59.6	74
76	67.5	32.7	36.0	34.1	31.7	25.4	50.1	48.6	45.4	61.3	76
78	69.3	33.6	37.0	35.0	32.6	26.3	51.5	49.9	46.6	63.0	78
80	71.1	34.4	38.0	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
82	72.8	35.3	38.9	36.8	34.5	28.2	54.2	52.6	49.0	66.3	82
84	74.6	36.2	39.9	37.7	35.4	29.1	55.6	53.9	50.1	68.0	84
86	76.4	37.1	40.9	38.6	36.3	30.0	57.0	55.3	51.3	69.7	86
88	78.2	38.0	41.8	39.5	37.2	31.0	58.4	56.6	52.5	71.3	88

<sup>1</sup> U. S. Bur. Standards Circ. 44, p. 139. The columns headed "Lactose" and "Lactose and Sucrose" were taken from "Methods of Sugar Analysis and Allied Determinations" by Arthur Given. The levulose equivalents are those of L. D. Hammond, *J. Research, Nat. Bur. Standards*, 24, 579 (1940), RP1301.

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROROSE (D-GLUCOSE)	LEVULOSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
					0.4 gram total sugar	2 grams total sugar		1 lactose, 4 su- crose	1 lactose, 12 su- crose		
90	79.9	38.9	42.8	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
92	81.7	39.8	43.8	41.4	39.1	32.8	61.1	59.3	54.9	74.7	92
94	83.5	40.6	44.8	42.3	40.0	33.8	62.5	60.6	56.0	76.3	94
96	85.3	41.5	45.7	43.2	41.0	34.7	63.8	61.9	57.2	78.0	96
98	87.1	42.4	46.7	44.1	41.9	35.6	65.2	63.3	58.4	79.7	98
100	88.8	43.3	47.7	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
102	90.6	44.2	48.6	46.0	43.8	37.5	68.0	66.0	60.8	83.0	102
104	92.4	45.1	49.6	46.9	44.7	38.5	69.3	67.3	62.0	84.7	104
106	94.2	46.0	50.6	47.8	45.6	39.4	70.7	68.6	63.2	86.3	106
108	95.9	46.9	51.6	48.7	46.6	40.3	72.1	70.0	64.4	88.0	108
110	97.7	47.8	52.6	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
112	99.5	48.7	53.5	50.6	48.4	42.2	74.8	72.6	66.7	91.3	112
114	101.3	49.6	54.5	51.5	49.4	43.2	76.2	74.0	67.9	93.0	114
116	103.0	50.5	55.5	52.4	50.3	44.1	77.6	75.3	69.1	94.7	116
118	104.8	51.4	56.5	53.3	51.2	45.0	79.0	76.7	70.3	96.4	118
120	106.6	52.3	57.5	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
122	108.4	53.2	58.4	55.2	53.1	46.9	81.7	79.3	72.7	99.7	122
124	110.1	54.1	59.4	56.1	54.1	47.9	83.1	80.7	73.9	101.4	124
126	111.9	55.0	60.4	57.0	55.0	48.8	84.5	82.0	75.1	103.0	126
128	113.7	55.9	61.4	58.0	55.9	49.8	85.8	83.4	76.3	104.7	128
130	115.5	56.8	62.4	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
132	117.2	57.7	63.4	59.8	57.8	51.7	88.6	86.0	78.7	108.0	132
134	119.0	58.6	64.3	60.8	58.8	52.6	90.0	87.4	79.7	109.7	134
136	120.8	59.5	65.3	61.7	59.7	53.6	91.3	88.7	81.1	111.4	136
138	122.6	60.4	66.3	62.6	60.7	54.5	92.7	90.1	82.3	113.0	138
140	124.4	61.3	67.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
142	126.1	62.2	68.3	64.5	62.6	56.4	95.5	92.8	84.7	116.4	142
144	127.9	63.1	69.3	65.4	63.5	57.4	96.8	94.1	85.9	118.0	144
146	129.7	64.0	70.3	66.4	64.5	58.3	98.2	95.4	87.1	119.7	146
148	131.5	65.0	71.3	67.3	65.4	59.3	99.6	96.8	88.3	121.4	148
150	133.2	65.9	72.2	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
152	135.0	66.8	73.2	69.2	67.3	61.2	102.3	99.5	90.8	124.7	152
154	136.8	67.7	74.2	70.1	68.3	62.1	103.7	100.8	92.0	126.4	154
156	138.6	68.6	75.2	71.1	69.2	63.1	105.1	102.2	93.2	128.0	156
158	140.3	69.5	76.2	72.0	70.2	64.1	106.5	103.5	94.4	129.7	158
160	142.1	70.4	77.2	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
162	143.9	71.4	78.2	73.9	72.1	66.0	109.2	106.2	96.8	133.0	162
164	145.7	72.3	79.2	74.9	73.1	66.9	110.6	107.5	98.0	134.7	164
166	147.4	73.2	80.2	75.8	74.0	67.9	112.0	108.9	99.2	136.4	166
168	149.2	74.1	81.2	76.8	75.0	68.8	113.4	110.2	100.4	138.0	168

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROSE (d-GLUCOSE)	LEVULOSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
					0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
170	151.0	75.1	82.2	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
172	152.8	76.0	83.2	78.7	76.9	70.8	116.1	112.9	102.8	141.4	172
174	154.6	76.9	84.2	79.6	77.9	71.7	117.5	114.3	104.1	143.0	174
176	156.3	77.8	85.2	80.6	78.8	72.7	118.9	115.6	105.3	144.7	176
178	158.1	78.8	86.2	81.5	79.8	73.7	120.3	117.0	106.5	146.4	178
180	159.9	79.7	87.2	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
182	161.7	80.6	88.2	83.4	81.7	75.6	123.1	119.7	108.9	149.7	182
184	163.4	81.5	89.2	84.4	82.7	76.6	124.3	121.0	110.1	151.4	184
186	165.2	82.5	90.2	85.3	83.7	77.6	125.8	122.4	111.3	153.0	186
188	167.0	83.4	91.2	86.3	84.6	78.5	127.2	123.7	112.5	154.7	188
190	168.8	84.3	92.2	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
192	170.5	85.3	93.2	88.2	86.6	80.5	129.9	126.4	115.0	158.0	192
194	172.3	86.2	94.2	89.2	87.6	81.4	131.3	127.8	116.2	159.7	194
196	174.1	87.1	95.2	90.1	88.5	82.4	132.7	129.2	117.4	161.4	196
198	175.9	88.1	96.2	91.1	89.5	83.4	134.1	130.5	118.6	163.0	198
200	177.6	89.0	97.2	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
202	179.4	89.9	98.3	93.0	91.4	85.3	136.8	133.2	121.0	166.4	202
204	181.2	90.9	99.3	94.0	92.4	86.3	138.2	134.6	122.3	168.0	204
206	183.0	91.8	100.3	94.9	93.4	87.3	139.6	135.9	123.5	169.7	206
208	184.7	92.8	101.3	95.9	94.4	88.3	141.0	137.3	124.7	171.4	208
210	186.5	93.7	102.3	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
212	188.3	94.6	103.3	97.8	96.3	90.2	143.7	140.0	127.2	174.7	212
214	190.1	95.6	104.3	98.8	97.3	91.2	145.1	141.4	128.4	176.4	214
216	191.9	96.5	105.3	99.8	98.3	92.2	146.5	142.7	129.6	178.0	216
218	193.6	97.5	106.4	100.8	99.3	93.2	147.9	144.1	130.9	179.7	218
220	195.4	98.4	107.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
222	197.2	99.4	108.4	102.7	101.2	95.1	150.7	146.8	133.3	183.0	222
224	199.0	100.3	109.4	103.7	102.2	96.1	152.0	148.1	134.5	184.7	224
226	200.7	101.3	110.4	104.6	103.2	97.1	153.4	149.5	135.8	186.4	226
228	202.5	102.2	111.4	105.6	104.2	98.1	154.8	150.8	137.0	188.0	228
230	204.3	103.2	112.5	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
232	206.1	104.1	113.5	107.6	106.2	100.1	157.6	153.6	139.4	191.3	232
234	207.8	105.1	114.5	108.6	107.2	101.1	159.0	154.9	140.7	193.0	234
236	209.6	106.0	115.5	109.5	108.2	102.1	160.3	156.3	141.9	194.7	236
238	211.4	107.0	116.5	110.5	109.2	103.1	161.7	157.6	143.2	196.3	238
240	213.2	108.0	117.6	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
242	214.9	108.9	118.6	112.5	111.1	105.0	164.5	160.3	145.6	199.7	242
244	216.7	109.9	119.6	113.5	112.1	106.0	165.9	161.7	146.9	201.3	244
246	218.5	110.8	120.6	114.5	113.1	107.0	167.3	163.1	148.1	203.0	246
248	220.3	111.8	121.7	115.4	114.1	108.0	168.7	164.4	149.3	204.7	248

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROSE (d-glucose)	LEVULOSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
					0.4 gram total sugar	2 grams total sugar		1 lactose, 4 su- crose	1 lactose, 12 su- crose		
250	222.1	112.8	122.7	116.4	115.1	109.0	170.1	165.8	150.6	206.3	250
252	223.8	113.7	123.7	117.4	116.1	110.0	171.5	167.2	151.8	208.0	252
254	225.6	114.7	124.7	118.4	117.1	111.0	172.8	168.5	153.1	209.7	254
256	227.4	115.7	125.8	119.4	118.1	112.0	174.2	169.9	154.3	211.3	256
258	229.2	116.6	126.8	120.4	119.1	113.0	175.6	171.3	155.5	213.0	258
260	230.9	117.6	127.8	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
262	232.7	118.6	128.9	122.4	121.1	115.0	178.4	174.0	158.0	216.3	262
264	234.5	119.5	129.9	123.4	122.1	116.0	179.8	175.3	159.3	218.0	264
266	236.3	120.5	130.9	124.4	123.1	117.0	181.2	176.7	160.5	219.7	266
268	238.0	121.5	131.9	125.4	124.1	118.0	182.6	178.1	161.8	221.3	268
270	239.8	122.5	133.0	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
272	241.6	123.4	134.0	127.4	126.2	120.0	185.3	180.8	164.3	224.6	272
274	243.4	124.4	135.0	128.4	127.2	121.1	186.7	182.2	165.5	226.3	274
276	245.1	125.4	136.1	129.4	128.2	122.1	188.1	183.5	166.8	228.0	276
278	246.9	126.4	137.1	130.4	129.2	123.1	189.5	184.9	168.0	229.6	278
280	248.7	127.3	138.2	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
282	250.5	128.3	139.2	132.4	131.2	125.1	192.3	187.6	170.5	233.0	282
284	252.3	129.3	140.2	133.4	132.2	126.1	193.7	189.0	171.8	234.6	284
286	254.0	130.3	141.3	134.4	133.2	127.1	195.1	190.4	173.0	236.3	286
288	255.8	131.3	142.3	135.4	134.3	128.1	196.5	191.7	174.3	238.0	288
290	257.6	132.3	143.4	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
292	259.4	133.2	144.4	137.4	136.3	130.2	199.2	194.4	176.8	241.3	292
294	261.1	134.2	145.4	138.4	137.3	131.2	200.6	195.8	178.1	242.9	294
296	262.9	135.2	146.5	139.4	138.3	132.2	202.0	197.2	179.3	244.6	296
298	264.7	136.2	147.5	140.5	139.4	133.2	203.4	198.6	180.6	246.3	298
300	266.5	137.2	148.6	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
302	268.2	138.2	149.6	142.5	141.4	135.3	206.2	201.3	183.1	249.6	302
304	270.0	139.2	150.6	143.5	142.4	136.3	207.6	202.7	184.4	251.3	304
306	271.8	140.2	151.7	144.5	143.4	137.3	209.0	204.0	185.6	252.9	306
308	273.6	141.2	152.8	145.5	144.5	138.3	210.4	205.4	186.9	254.6	308
310	275.3	142.2	153.8	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
312	277.1	143.2	154.9	147.6	146.5	140.4	213.2	208.1	189.4	257.9	312
314	278.9	144.2	155.9	148.6	147.6	141.4	214.6	209.5	190.7	259.6	314
316	280.7	145.2	157.0	149.6	148.6	142.4	216.0	210.9	191.9	261.2	316
318	282.5	146.2	158.0	150.7	149.6	143.5	217.3	212.2	193.2	262.9	318
320	284.2	147.2	159.1	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
322	286.0	148.2	160.1	152.7	151.7	145.5	220.1	215.5	195.7	266.2	322
324	287.8	149.2	161.2	153.7	152.7	146.6	221.5	216.4	197.0	267.9	324
326	289.6	150.2	162.2	154.8	153.8	147.6	222.9	217.7	198.2	269.6	326
328	291.3	151.2	163.3	155.8	154.8	148.6	224.3	219.1	199.5	271.2	328

9

Munson and Walker's table.—Continued.

(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROROSE (d-glucose)	LEVULOSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
					0.4 gram total sugar	2 grams total sugar		1 lactose, 4 su- crose	1 lactose, 12 su- crose		
330	293.1	152.2	164.3	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
332	294.9	153.2	165.4	157.9	156.9	150.7	227.1	221.8	202.0	274.6	332
334	296.7	154.2	166.4	158.9	157.9	151.7	228.5	223.2	203.3	276.2	334
336	298.4	155.2	167.5	159.9	159.0	152.8	229.9	224.6	204.6	277.9	336
338	300.2	156.3	168.6	161.0	160.0	153.8	231.3	226.0	205.9	279.5	338
340	302.0	157.3	169.6	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
342	303.8	158.3	170.7	163.1	162.1	155.9	234.1	228.7	208.4	282.9	342
344	305.5	159.3	171.7	164.1	163.1	156.9	235.5	230.1	209.7	284.5	344
346	307.3	160.3	172.8	165.1	164.2	158.0	236.9	231.5	211.0	286.2	346
348	309.1	161.4	173.9	166.2	165.2	159.0	238.3	232.9	212.2	287.9	348
350	310.9	162.4	174.9	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
352	312.7	163.4	176.0	168.3	167.3	161.1	241.1	235.6	214.8	291.2	352
354	314.4	164.4	177.1	169.3	168.4	162.2	242.5	237.0	216.1	292.8	354
356	316.2	165.4	178.1	170.4	169.4	163.2	243.9	238.4	217.3	294.5	356
358	318.0	166.5	179.2	171.4	170.5	164.3	245.3	239.8	218.6	296.2	358
360	319.8	167.5	180.2	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
362	321.5	168.5	181.3	173.5	172.6	166.4	248.1	242.5	221.2	299.5	362
364	323.3	169.6	182.4	174.6	173.7	167.4	249.5	243.9	222.5	301.2	364
366	325.1	170.6	183.5	175.6	174.7	168.5	250.9	245.3	223.7	302.8	366
368	326.9	171.6	184.5	176.7	175.8	169.5	252.3	246.7	225.0	304.5	368
370	328.6	172.7	185.6	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
372	330.4	173.7	186.7	178.8	177.9	171.6	255.1	249.5	227.6	307.8	372
374	332.2	174.7	187.7	179.8	179.0	172.7	256.5	250.9	228.9	309.5	374
376	334.0	175.8	188.8	180.9	180.0	173.7	257.9	252.2	230.2	311.1	376
378	335.7	176.8	189.9	182.0	181.1	174.8	259.3	253.6	231.5	312.8	378
380	337.5	177.9	191.0	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
382	339.3	178.9	192.1	184.1	183.2	176.9	262.1	256.4	234.1	316.1	382
384	341.1	180.0	193.1	185.2	184.3	178.0	263.5	257.8	235.4	317.8	384
386	342.9	181.0	194.2	186.2	185.4	179.1	264.9	259.2	236.6	319.4	386
388	344.6	182.0	195.3	187.3	186.4	180.1	266.5	260.5	237.9	321.1	388
390	346.4	183.1	196.4	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
392	348.2	184.1	197.4	189.4	188.6	182.3	269.1	263.3	240.5	324.4	392
394	350.0	185.2	198.5	190.5	189.7	183.3	270.5	264.7	241.8	326.1	394
396	351.7	186.2	199.6	191.6	190.7	184.4	271.9	266.1	243.1	327.7	396
398	353.5	187.3	200.7	192.7	191.8	185.5	273.3	267.5	244.4	329.4	398
400	355.3	188.4	201.8	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
402	357.1	189.4	202.9	194.8	194.0	187.6	276.1	270.3	247.0	332.7	402
404	358.8	190.5	204.0	195.9	195.0	188.7	277.5	271.7	248.3	334.4	404
406	360.6	191.5	205.0	197.0	196.1	189.8	278.9	273.0	249.6	336.0	406
408	362.4	192.6	206.1	198.1	197.2	190.8	280.3	274.4	251.0	337.7	408

*Munson and Walker's table.—Concluded.*  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	COPPER (Cu)	DEXTROSE (d-GLUCOSE)	LEVULOSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu <sub>2</sub> O)
					0.4 gram total sugar	2 grams total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	1 lactose, 4 su- crose	1 lactose, 12 su- crose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	
410	364.2	193.7	207.2	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
412	365.9	194.7	208.3	200.2	199.4	193.0	283.2	277.2	253.6	341.0	412
414	367.7	195.8	209.4	201.3	200.5	194.1	284.6	278.6	254.9	342.7	414
416	369.5	196.8	210.5	202.4	201.6	195.2	286.0	280.0	256.2	344.4	416
418	371.3	197.9	211.6	203.5	202.6	196.2	287.4	281.4	257.5	346.0	418
420	373.1	199.0	212.7	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
422	374.8	200.1	213.8	205.7	204.8	198.4	290.2	284.2	260.1	349.3	422
424	376.6	201.1	214.9	206.7	205.9	199.5	291.6	285.6	261.4	351.0	424
426	378.4	202.2	216.0	207.8	207.0	200.6	293.0	287.0	262.7	352.7	426
428	380.2	203.3	217.1	208.9	208.1	201.7	294.4	288.4	264.0	354.3	428
430	381.9	204.4	218.2	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
432	383.7	205.5	219.3	211.1	210.3	203.8	297.2	291.2	266.6	357.6	432
434	385.5	206.5	220.4	212.2	211.4	204.9	298.6	292.6	268.0	359.3	434
436	387.3	207.6	221.5	213.3	212.5	206.0	300.0	294.0	269.3	361.0	436
438	389.1	308.7	222.6	214.4	213.6	207.1	301.4	295.4	270.6	362.6	438
440	390.8	209.8	223.7	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
442	392.6	210.9	224.8	216.6	215.8	209.3	304.2	298.2	273.3	365.9	442
444	394.4	212.0	225.9	217.8	216.9	210.4	305.6	299.6	274.6	367.6	444
446	396.1	213.1	227.0	218.9	218.0	211.5	307.0	301.0	275.9	369.3	446
448	397.9	214.1	228.1	220.0	219.1	212.6	308.4	302.4	277.2	370.9	448
450	399.7	215.2	229.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
452	401.5	216.3	230.4	222.2	221.4	214.8	311.3	305.2	279.9	374.2	452
454	403.3	217.4	231.5	223.3	222.5	215.9	312.7	306.6	281.2	375.9	454
456	405.0	218.5	232.6	224.4	223.6	217.0	314.1	308.0	282.5	377.6	456
458	406.8	219.6	233.7	225.5	224.7	218.1	315.5	309.4	283.9	379.2	458
460	408.6	220.7	234.9	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
462	410.4	221.8	236.0	227.8	226.9	220.3	318.3	312.2	286.5	382.5	462
464	412.1	222.9	237.2	228.9	228.1	221.4	319.7	313.6	287.8	384.2	464
466	413.9	224.0	238.3	230.0	229.2	222.5	321.1	315.0	289.2	385.9	466
468	415.7	225.1	239.5	231.2	230.3	223.7	322.5	316.4	290.5	387.5	468
470	417.5	226.2	240.6	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
472	419.2	227.4	241.8	233.4	232.5	225.9	325.3	319.1	293.2	390.8	472
474	421.0	228.3	242.9	234.5	233.7	227.0	326.8	320.5	294.5	392.5	474
476	422.8	229.6	244.1	235.7	234.8	228.1	328.2	321.9	295.8	394.2	476
478	424.6	230.7	245.3	236.8	235.9	229.2	329.6	323.3	297.1	395.8	478
480	426.3	231.8	246.6	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
482	428.1	232.9	247.9	239.1	238.2	231.5	332.4	326.1	299.8	399.1	482
484	429.9	234.1	249.1	240.2	239.3	232.6	333.8	327.5	301.1	400.8	484
486	431.7	235.2	250.6	241.4	240.5	233.7	335.2	328.9	302.5	402.4	486
488	433.5	236.3	252.1	242.5	241.6	234.8	336.6	330.3	303.8	404.1	488
490	435.2	237.4	253.9	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

10 *Herzfeld's table for determining invert sugar in raw sugars (invert sugar not to exceed 1.5 per cent).*<sup>1</sup>

COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR
mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
50	0.050	110	0.351	170	0.680	230	1.013	290	1.357
52	.058	112	.361	172	.692	232	.024	292	.368
54	.066	114	.371	174	.704	234	.036	294	.380
56	.074	116	.381	176	.715	236	.047	296	.391
58	.082	118	.392	178	.726	238	.058	298	.403
60	.090	120	.402	180	.737	240	.070	300	.414
62	.098	122	.412	182	.748	242	.081	302	.425
64	.108	124	.423	184	.759	244	.093	304	.437
66	.118	126	.433	186	.770	246	.104	306	.448
68	.128	128	.443	188	.781	248	.116	308	.460
70	.138	130	.453	190	.792	250	.127	310	.471
72	.148	132	.463	192	.803	252	.139	312	.483
74	.157	134	.473	194	.814	254	.150	314	.494
76	.167	136	.483	196	.825	256	.162		
78	.177	138	.493	198	.836	258	.173		
80	.187	140	.503	200	.847	260	.185		
82	.197	142	.515	202	.858	262	.196		
84	.208	144	.527	204	.869	264	.207		
86	.219	146	.538	206	.880	266	.219		
88	.231	148	.550	208	.891	268	.231		
90	.242	150	.562	210	.902	270	.242		
92	.254	152	.574	212	.913	272	.253		
94	.265	154	.586	214	.924	274	.265		
96	.277	156	.598	216	.935	276	.276		
98	.288	158	.609	218	.946	278	.288		
100	.300	160	.621	220	.957	280	.299		
102	.310	162	.633	222	.968	282	.311		
104	.320	164	.645	224	.979	284	.322		
106	.330	166	.657	226	.990	286	.334		
108	.340	168	.669	228	1.001	288	.345		

<sup>1</sup> *Z. Ver. Rucbenzucker-Ind.*, 35 (N.F. 22); 1012 (1885).



*Meissl and Hiller's factors for determining invert sugar in materials in which, of 11*  
*the total sugars present, more than 1.5 per cent is invert sugar, and less*  
*than 98.5 per cent is sucrose.<sup>1</sup>*

RATIO OF SUCROSE TO INVERT SUGAR = R:I	APPROXIMATE ABSOLUTE WEIGHT OF INVERT SUGAR (Z)						
	200 mg	175 mg	150 mg	125 mg	100 mg	75 mg	50 mg
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0:100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10:90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20:80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30:70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40:60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50:50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60:40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70:30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80:20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90:10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91:9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92:8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93:7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94:6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95:5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96:4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97:3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98:2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99:1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

<sup>1</sup> *Z. Ver. Rucbenzucker-Ind.*, 39 (N.F. 26), 734, (1889).

Wein's table for the determination of mallose.<sup>1</sup>

(Expressed in milligrams.)

COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE
32	36.0	27.0	122	137.4	106.2	212	238.7	186.8
34	38.3	28.7	124	139.6	108.0	214	240.9	188.6
36	40.5	30.5	126	141.9	109.8	216	243.2	190.4
38	42.8	32.2	128	144.1	111.6	218	245.4	192.1
40	45.0	33.9	130	146.4	113.4	220	247.7	193.9
42	47.3	35.7	132	148.6	115.2	222	249.9	195.7
44	49.5	37.4	134	150.9	117.0	224	252.4	197.5
46	51.8	39.1	136	153.1	118.8	226	254.4	199.3
48	54.0	40.9	138	155.4	120.6	228	256.7	201.1
50	56.3	42.6	140	157.6	122.4	230	258.9	202.9
52	58.5	44.4	142	159.9	124.2	232	261.2	204.7
54	60.8	46.1	144	162.1	126.0	234	263.4	206.5
56	63.0	47.8	146	164.4	127.8	236	265.7	208.3
58	65.3	49.6	148	166.6	129.6	238	268.0	210.0
60	67.6	51.3	150	168.9	131.4	240	270.2	211.8
62	69.8	53.1	152	171.1	133.2	242	272.5	213.6
64	72.1	54.8	154	173.4	135.0	244	274.7	215.4
66	74.3	56.6	156	175.6	136.8	246	277.0	217.2
68	76.6	58.3	158	177.9	138.6	248	279.2	219.0
70	78.8	60.1	160	180.1	140.4	250	281.5	220.8
72	81.1	61.8	162	182.4	142.2	252	283.7	222.6
74	83.3	63.6	164	184.6	144.0	254	286.0	224.4
76	85.6	65.4	166	186.9	145.8	256	288.2	226.2
78	87.8	67.1	168	189.1	147.8	258	290.5	228.0
80	90.1	68.9	170	191.4	149.4	260	292.7	229.8
82	92.3	70.6	172	193.6	151.2	262	295.0	231.6
84	94.6	72.4	174	195.9	152.9	264	297.2	233.4
86	96.8	74.1	176	198.1	154.7	266	299.5	235.2
88	99.1	75.9	178	200.4	156.5	268	301.7	237.0
90	101.3	77.7	180	202.6	158.3	270	304.0	238.8
92	103.6	79.5	182	204.9	160.1	272	306.2	240.6
94	105.8	81.2	184	207.1	161.8	274	308.5	242.4
96	108.1	83.0	186	209.4	163.6	276	310.7	244.2
98	110.3	84.8	188	211.7	165.4	278	313.0	246.0
100	112.6	86.6	190	213.9	167.2	280	315.2	247.8
102	114.8	88.4	192	216.2	169.0	282	317.5	249.6
104	117.1	90.1	194	218.4	170.7	284	319.7	251.3
106	119.3	91.9	196	220.7	172.5	286	322.0	253.1
108	121.6	93.7	198	222.9	174.3	288	324.2	254.9
110	123.8	95.5	200	225.2	176.1	290	326.5	256.6
112	126.1	97.3	202	227.4	177.9	292	328.7	258.4
114	128.3	99.0	204	229.7	179.6	294	331.0	260.2
116	130.6	100.8	206	231.9	181.4	296	333.2	262.0
118	132.8	102.6	208	234.2	183.2	298	335.5	263.7
120	135.1	104.4	210	236.4	185.0	300	337.8	265.5

<sup>1</sup> Tables for the Quantitative Estimation of the Sugars. Translated by Frew, 1896, p. 26.

*Copper-levulose equivalents according to Jackson and Mathews' modification of Nyns' selective method for levulose.*

(Expressed in milligrams. A linear interpolation yields accurate results.)

Cu	LEVULOSE	Cu	LEVULOSE
5	2.5	130	39.3
10	4.5	140	42.0
15	6.2	150	44.7
20	7.9	160	47.4
25	9.5	170	50.0
30	11.0	180	52.6
35	12.5	190	55.2
40	13.9	200	57.9
45	15.4	210	60.6
50	16.8	220	63.4
55	18.3	230	66.4
60	19.7	240	69.4
65	21.2	250	72.5
70	22.5	260	75.7
80	25.4	270	79.0
90	28.1	280	82.4
100	30.9	290	85.9
110	33.7	300	89.5
120	36.5	310	93.2

14

*Allihn's table for the determination of dextrose.*<sup>1</sup>  
(Expressed in milligrams.)

COPPER	CUPROUS OXIDE	DEXTROSE	COPPER	CUPROUS OXIDE	DEXTROSE	COPPER	CUPROUS OXIDE	DEXTROSE
12	13.5	7.1	102	114.8	51.9	192	216.2	98.4
14	15.8	8.1	104	117.1	52.9	194	218.4	99.4
16	18.0	9.0	106	119.3	54.0	196	220.7	100.5
18	20.3	10.0	108	121.6	55.0	198	222.9	101.5
20	22.5	11.0	110	123.8	56.0	200	225.2	102.6
22	24.8	12.0	112	126.1	57.0	202	227.4	103.7
24	27.0	13.0	114	128.3	58.0	204	229.7	104.7
26	29.3	14.0	116	130.6	59.1	206	231.9	105.8
28	31.5	15.0	118	132.8	60.1	208	234.2	106.8
30	33.8	16.0	120	135.1	61.1	210	236.4	107.9
32	36.0	17.0	122	137.4	62.1	212	238.7	109.0
34	38.3	18.0	124	139.6	63.1	214	240.9	110.0
36	40.5	18.9	126	141.9	64.2	216	243.2	111.1
38	42.8	19.9	128	144.1	65.2	218	245.4	112.1
40	45.0	20.9	130	146.4	66.2	220	247.7	113.2
42	47.3	21.9	132	148.6	67.2	222	249.9	114.3
44	49.5	22.9	134	150.9	68.2	224	252.4	115.3
46	51.8	23.9	136	153.1	69.3	226	254.4	116.4
48	54.0	24.9	138	155.4	70.3	228	256.7	117.4
50	56.3	25.9	140	157.6	71.3	230	258.9	118.5
52	58.5	26.9	142	159.9	72.3	232	261.2	119.6
54	60.8	27.9	144	162.1	73.4	234	263.4	120.7
56	63.0	28.8	146	164.4	74.4	236	265.7	121.7
58	65.3	29.8	148	166.6	75.5	238	268.0	122.8
60	67.6	30.8	150	168.9	76.5	240	270.2	123.9
62	69.8	31.8	152	171.1	77.5	242	272.5	125.0
64	72.1	32.8	154	173.4	78.6	244	274.7	126.0
66	74.3	33.8	156	175.6	79.6	246	277.0	127.1
68	76.6	34.8	158	177.9	80.7	248	279.2	128.1
70	78.8	35.8	160	180.1	81.7	250	281.5	129.2
72	81.1	36.8	162	182.4	82.6	252	283.7	130.3
74	83.3	37.8	164	184.6	83.7	254	286.0	131.4
76	85.6	38.8	166	186.9	84.8	256	288.2	132.4
78	87.8	39.8	168	189.1	85.9	258	290.5	133.5
80	90.1	40.8	170	191.4	86.9	260	292.7	134.6
82	92.3	41.8	172	193.6	87.9	262	295.0	135.7
84	94.6	42.8	174	195.9	89.0	264	297.2	136.8
86	96.8	43.9	176	198.1	90.0	266	299.5	137.8
88	99.1	44.9	178	200.4	91.1	268	301.7	138.9
90	101.3	45.9	180	202.6	92.1	270	304.0	140.0
92	103.6	46.9	182	204.9	93.1	272	306.2	141.1
94	105.8	47.9	184	207.1	94.2	274	308.5	142.2
96	108.1	48.9	186	209.4	95.2	276	310.7	143.3
98	110.3	49.9	188	211.7	96.3	278	313.0	144.4
100	112.6	50.9	190	213.9	97.3	280	315.2	145.5

<sup>1</sup> Z. Ver. Ruebenzucker-Ind., 32 (N.F. 19), 606, 865 (1882).

*Allihn's table.*—Concluded.

14

(Expressed in milligrams.)

COPPER	CUPROUS OXIDE	DEXTROSE	COPPER	CUPROUS OXIDE	DEXTROSE	COPPER	CUPROUS OXIDE	DEXTROSE
282	317.5	146.6	342	385.0	179.8	402	452.6	214.1
284	319.7	147.7	344	387.3	180.9	404	454.8	215.2
286	322.0	148.8	346	389.6	182.1	406	457.1	216.4
288	324.2	149.9	348	391.8	183.2	408	459.4	217.5
290	326.5	151.0	350	394.0	184.3	410	461.6	218.7
292	328.7	152.1	352	396.3	185.4	412	463.8	219.9
294	331.0	153.2	354	398.6	186.6	414	466.1	221.0
296	333.3	154.3	356	400.8	187.7	416	468.4	222.2
298	335.5	155.4	358	403.1	188.9	418	470.6	223.3
300	337.8	156.5	360	405.3	190.0	420	472.9	224.5
302	340.0	157.6	362	407.6	191.1	422	475.6	225.7
304	342.3	158.7	364	409.8	192.3	424	477.4	226.9
306	344.5	159.8	366	412.1	193.4	426	479.6	228.0
308	346.8	160.9	368	414.3	194.6	428	481.9	229.2
310	349.0	162.0	370	416.6	195.7	430	484.1	230.4
312	351.3	163.1	372	418.8	196.8	432	486.4	231.6
314	353.5	164.2	374	421.1	198.0	434	488.6	232.8
316	355.8	165.3	376	423.3	199.1	436	490.9	233.9
318	358.0	166.4	378	425.6	200.3	438	493.1	235.1
320	360.3	167.5	380	427.8	201.4	440	495.4	236.3
322	362.5	168.6	382	430.1	202.5	442	497.6	237.5
324	364.8	169.7	384	432.3	203.7	444	499.9	238.7
326	367.0	170.9	386	434.6	204.8	446	502.1	239.8
328	369.3	172.0	388	436.8	206.0	448	504.4	241.0
330	371.5	173.1	390	439.1	207.1	450	506.6	242.2
332	373.8	174.2	392	441.3	208.3	452	508.9	243.4
334	376.0	175.3	394	443.6	209.4	454	511.1	244.6
336	378.3	176.5	396	445.9	210.6	456	513.4	245.7
338	380.5	177.6	398	448.1	211.7	458	515.6	246.9
340	382.8	178.7	400	450.3	212.9	460	517.9	248.1
						462	520.1	249.3

15

*Factors for 10 ml Soxhlet's solution to be used in connection with the Lane-Eynon general volumetric method.*

TITER	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	5 GRAMS SUCROSE PER 100 ML INVERT SUGAR	10 GRAMS SUCROSE PER 100 ML INVERT SUGAR	25 GRAMS SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

*Factors for 25 ml Soxhlet's solution to be used in connection with the Lane-Eynon general volumetric method.*

TITER	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9
32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

17 *Quisumbing and Thomas table for calculating dextrose, levulose, invert sugar, lactose, and maltose.*  
(Expressed in milligrams.)

COPPER (Cu)	CUPROUS OXIDE (Cu <sub>2</sub> O)	DEXTROROSE (d-GLUCOSE)	LEVULOROSE (d-FRUCTOSE)	INVERT SUGAR	LACTOSE		MALTOSE	
					C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> · H <sub>2</sub> O	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> · H <sub>2</sub> O
10	11.1	4.8	5.3	5.0	7.7	8.1	9.4	9.9
20	22.5	9.5	10.5	10.1	15.5	16.3	18.8	19.8
30	33.8	14.3	15.8	15.2	23.2	24.4	28.2	29.7
40	45.0	19.1	21.2	20.3	30.9	32.5	37.6	39.6
50	56.3	24.0	26.5	25.4	38.7	40.7	47.0	49.5
60	67.6	28.9	31.9	30.6	46.4	48.8	56.4	59.4
70	78.8	33.7	37.2	35.7	54.0	56.9	65.8	69.3
80	90.1	38.7	42.6	40.9	61.7	65.0	75.2	79.2
90	101.3	43.6	48.0	46.1	69.5	73.2	84.6	89.1
100	112.6	48.6	53.4	51.3	77.2	81.3	94.0	99.0
110	123.8	53.5	58.8	56.5	85.0	89.5	103.4	108.9
120	135.1	58.5	64.3	61.8	92.7	97.0	112.8	118.8
130	146.4	63.5	70.7	67.0	100.4	105.7	122.2	128.7
140	157.6	68.6	75.2	72.3	108.2	113.9	131.6	138.6
150	168.9	73.7	80.7	77.6	116.0	122.0	141.0	148.5
160	180.1	78.8	86.2	82.9	123.7	130.1	150.4	158.4
170	191.4	83.9	91.7	88.3	131.4	138.3	159.8	168.3
180	202.6	89.1	97.2	93.7	139.1	146.4	169.2	178.2
190	213.9	94.2	102.8	99.1	146.9	154.6	178.8	188.1
200	225.2	99.4	108.4	104.4	154.6	162.7	188.2	198.0
210	236.4	104.6	114.0	109.8	162.3	170.9	197.6	207.9
220	247.7	109.9	119.6	115.2	170.0	179.0	207.0	217.8
230	258.9	115.1	125.2	120.6	177.8	187.2	216.4	227.7
240	270.2	120.4	130.8	126.1	185.5	195.3	225.8	237.6
250	281.5	125.7	136.4	131.6	193.2	203.4	235.2	247.5
260	292.7	131.0	142.1	137.1	201.0	211.6	244.6	257.4
270	304.0	136.4	147.8	142.6	208.8	219.8	254.0	267.3
280	315.2	141.7	153.5	148.2	216.5	227.9	263.4	277.2
290	326.5	147.1	159.2	153.7	224.2	236.0	272.8	287.1
300	337.8	152.6	165.0	159.3	232.0	244.2	282.2	297.0
310	349.0	158.0	170.7	164.9	239.7	252.3	291.6	306.9
320	360.3	163.5	176.5	170.5	247.5	260.5	301.0	316.8
330	371.5	168.9	182.3	176.1	255.3	268.7	310.4	326.7
340	382.8	174.5	188.1	181.8	263.0	276.8	319.8	336.6
350	394.0	180.0	193.9	187.4	270.7	285.0	329.2	346.5
360	405.3	185.5	199.7	193.1	278.4	293.1	338.6	356.4
370	416.6	191.1	205.5	198.8	286.2	301.3	348.0	366.3
380	427.8	196.7	211.4	204.5	293.9	309.4	357.4	376.2
390	439.1	202.3	217.3	210.2	301.6	317.5	366.8	386.1
400	450.3	208.0	223.2	216.0	309.4	325.7	376.2	396.0
410	461.6	213.7	229.1	221.8	317.1	333.8	385.6	405.9
420	472.9	219.4	235.0	227.6	324.9	342.0	395.0	415.8
430	484.1	225.1	240.9	233.4	332.6	350.1	404.4	425.7
440	495.4	230.8	246.9	239.2	340.4	358.3	413.8	435.6
450	506.6	236.6	252.9	245.0	348.1	366.4	423.2	445.5
460	517.9	242.4	258.9	250.9	355.9	374.6	432.6	455.4
470	529.1	248.1	264.9	256.8	363.6	382.7	442.0	465.3
480	540.4	250.8	270.9	262.7	371.3	390.9	451.4	475.2



*Kröber's table for the determination of pentoses and pentosans.*  
(Expressed in grams.)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
.032	.0193	.0413	.0363	.0342	.0301	.0378	.0333
.034	.0203	.0435	.0383	.0361	.0317	.0398	.0350
.036	.0214	.0457	.0402	.0379	.0334	.0418	.0368
.038	.0224	.0479	.0422	.0398	.0350	.0439	.0386
.040	.0235	.0501	.0441	.0416	.0366	.0459	.0404
.042	.0245	.0523	.0460	.0434	.0382	.0479	.0422
.044	.0255	.0545	.0480	.0452	.0398	.0499	.0440
.046	.0266	.0567	.0499	.0471	.0414	.0519	.0457
.048	.0276	.0589	.0519	.0489	.0430	.0539	.0475
.050	.0286	.0611	.0538	.0507	.0446	.0559	.0492
.052	.0297	.0633	.0557	.0525	.0462	.0579	.0510
.054	.0307	.0655	.0576	.0543	.0478	.0599	.0528
.056	.0318	.0677	.0596	.0562	.0494	.0620	.0546
.058	.0328	.0699	.0615	.0580	.0510	.0640	.0564
.060	.0338	.0721	.0634	.0598	.0526	.0660	.0581
.062	.0349	.0743	.0653	.0616	.0542	.0680	.0599
.064	.0359	.0765	.0673	.0635	.0558	.0700	.0617
.066	.0370	.0787	.0692	.0653	.0575	.0720	.0634
.068	.0380	.0809	.0712	.0672	.0591	.0741	.0652
.070	.0390	.0831	.0731	.0690	.0607	.0761	.0670
.072	.0401	.0853	.0750	.0708	.0623	.0781	.0688
.074	.0411	.0875	.0770	.0726	.0639	.0801	.0706
.076	.0422	.0897	.0789	.0745	.0655	.0821	.0722
.078	.0432	.0919	.0809	.0763	.0671	.0841	.0740
.080	.0442	.0941	.0828	.0781	.0687	.0861	.0758
.082	.0453	.0963	.0847	.0799	.0703	.0881	.0776
.084	.0463	.0985	.0867	.0817	.0719	.0901	.0794
.086	.0474	.1007	.0886	.0836	.0735	.0922	.0812
.088	.0484	.1029	.0906	.0854	.0751	.0942	.0830
.090	.0494	.1051	.0925	.0872	.0767	.0962	.0847
.092	.0505	.1073	.0944	.0890	.0783	.0982	.0865
.094	.0515	.1095	.0964	.0909	.0800	.1002	.0883
.096	.0525	.1117	.0983	.0927	.0816	.1022	.0899
.098	.0536	.1139	.1003	.0946	.0832	.1043	.0917
.100	.0546	.1161	.1022	.0964	.0848	.1063	.0935
.102	.0557	.1182	.1041	.0982	.0864	.1083	.0953
.104	.0567	.1204	.1060	.1000	.0880	.1103	.0971
.106	.0577	.1226	.1080	.1019	.0896	.1123	.0988
.108	.0588	.1248	.1099	.1037	.0912	.1143	.1006
.110	.0598	.1270	.1118	.1055	.0928	.1163	.1023
.112	.0608	.1292	.1137	.1073	.0944	.1183	.1041
.114	.0619	.1314	.1156	.1091	.0960	.1203	.1059
.116	.0629	.1336	.1176	.1110	.0976	.1223	.1076
.118	.0640	.1358	.1195	.1128	.0992	.1243	.1094

18

*Kröber's table.*—Continued.  
(Expressed in grams.)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
.122	.0660	.1402	.1233	.1164	.1024	.1283	.1129
.124	.0671	.1424	.1253	.1182	.1040	.1303	.1147
.126	.0681	.1446	.1272	.1201	.1057	.1324	.1165
.128	.0691	.1468	.1292	.1219	.1073	.1344	.1183
.130	.0702	.1490	.1311	.1237	.1089	.1364	.1201
.132	.0712	.1512	.1330	.1255	.1105	.1384	.1219
.134	.0723	.1534	.1350	.1273	.1121	.1404	.1236
.136	.0733	.1556	.1369	.1292	.1137	.1424	.1253
.138	.0743	.1578	.1389	.1310	.1153	.1444	.1271
.140	.0754	.1600	.1408	.1328	.1169	.1464	.1288
.142	.0764	.1622	.1427	.1346	.1185	.1484	.1306
.144	.0774	.1644	.1447	.1364	.1201	.1504	.1324
.146	.0785	.1666	.1466	.1383	.1217	.1525	.1342
.148	.0795	.1688	.1486	.1401	.1233	.1545	.1360
.150	.0805	.1710	.1505	.1419	.1249	.1565	.1377
.152	.0816	.1732	.1524	.1437	.1265	.1585	.1395
.154	.0826	.1754	.1544	.1455	.1281	.1605	.1413
.156	.0837	.1776	.1563	.1474	.1297	.1625	.1430
.158	.0847	.1798	.1583	.1492	.1313	.1645	.1448
.160	.0857	.1820	.1602	.1510	.1329	.1665	.1465
.162	.0868	.1842	.1621	.1528	.1345	.1685	.1483
.164	.0878	.1864	.1640	.1546	.1361	.1705	.1501
.166	.0888	.1886	.1660	.1565	.1377	.1726	.1519
.168	.0899	.1908	.1679	.1583	.1393	.1746	.1537
.170	.0909	.1930	.1698	.1601	.1409	.1766	.1554
.172	.0920	.1952	.1717	.1619	.1425	.1786	.1572
.174	.0930	.1974	.1736	.1637	.1441	.1806	.1590
.176	.0940	.1996	.1756	.1656	.1457	.1826	.1607
.178	.0951	.2018	.1775	.1674	.1473	.1846	.1625
.180	.0961	.2039	.1794	.1692	.1489	.1866	.1642
.182	.0971	.2061	.1813	.1710	.1505	.1886	.1660
.184	.0982	.2082	.1832	.1728	.1521	.1906	.1678
.186	.0992	.2104	.1851	.1747	.1537	.1926	.1695
.188	.1003	.2126	.1870	.1765	.1553	.1946	.1712
.190	.1013	.2147	.1889	.1783	.1569	.1965	.1729
.192	.1023	.2168	.1908	.1801	.1585	.1985	.1747
.194	.1034	.2190	.1927	.1819	.1601	.2005	.1764
.196	.1044	.2212	.1946	.1838	.1617	.2025	.1782
.198	.1054	.2233	.1965	.1856	.1633	.2045	.1800
.200	.1065	.2255	.1984	.1874	.1649	.2065	.1817
.202	.1075	.2276	.2003	.1892	.1665	.2085	.1835
.204	.1085	.2298	.2022	.1910	.1681	.2105	.1853
.206	.1096	.2320	.2041	.1929	.1697	.2125	.1869
.208	.1106	.2341	.2060	.1947	.1713	.2144	.1887

*Kröber's table.*—Concluded.  
(Expressed in grams.)

18

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
.212	.1127	.2384	.2098	.1984	.1745	.2184	.1922
.214	.1137	.2406	.2117	.2002	.1761	.2204	.1940
.216	.1147	.2428	.2136	.2020	.1778	.2224	.1957
.218	.1158	.2449	.2155	.2038	.1794	.2244	.1974
.220	.1168	.2471	.2174	.2057	.1810	.2264	.1992
.222	.1178	.2492	.2193	.2075	.1826	.2284	.2010
.224	.1189	.2514	.2212	.2093	.1842	.2304	.2028
.226	.1199	.2536	.2232	.2111	.1858	.2324	.2046
.228	.1209	.2557	.2251	.2130	.1874	.2344	.2063
.230	.1220	.2579	.2270	.2148	.1890	.2364	.2081
.232	.1230	.2600	.2289	.2166	.1906	.2383	.2097
.234	.1240	.2622	.2308	.2184	.1922	.2403	.2115
.236	.1251	.2644	.2327	.2202	.1938	.2423	.2132
.238	.1261	.2665	.2346	.2220	.1954	.2443	.2150
.240	.1271	.2687	.2365	.2239	.1970	.2463	.2168
.242	.1281	.2708	.2384	.2257	.1986	.2483	.2185
.244	.1292	.2730	.2403	.2275	.2002	.2503	.2203
.246	.1302	.2752	.2422	.2293	.2018	.2523	.2220
.248	.1312	.2773	.2441	.2311	.2034	.2543	.2238
.250	.1323	.2795	.2460	.2330	.2050	.2563	.2256
.252	.1333	.2816	.2479	.2348	.2066	.2582	.2272
.254	.1343	.2838	.2498	.2366	.2082	.2602	.2290
.256	.1354	.2860	.2517	.2384	.2098	.2622	.2307
.258	.1364	.2881	.2536	.2402	.2114	.2642	.2325
.260	.1374	.2903	.2555	.2420	.2130	.2662	.2342
.262	.1385	.2924	.2574	.2438	.2146	.2681	.2359
.264	.1395	.2946	.2593	.2456	.2162	.2701	.2377
.266	.1405	.2968	.2612	.2474	.2178	.2721	.2394
.268	.1416	.2989	.2631	.2492	.2194	.2741	.2412
.270	.1426	.3011	.2650	.2511	.2210	.2761	.2429
.272	.1436	.3032	.2669	.2529	.2226	.2781	.2447
.274	.1447	.3054	.2688	.2547	.2242	.2801	.2465
.276	.1457	.3076	.2707	.2565	.2258	.2821	.2482
.278	.1467	.3097	.2726	.2583	.2274	.2840	.2499
.280	.1478	.3119	.2745	.2602	.2290	.2861	.2517
.282	.1488	.3140	.2764	.2620	.2306	.2880	.2534
.284	.1498	.3162	.2783	.2638	.2322	.2900	.2552
.286	.1509	.3184	.2802	.2656	.2338	.2920	.2570
.288	.1519	.3205	.2821	.2674	.2354	.2940	.2587
.290	.1529	.3227	.2840	.2693	.2370	.2960	.2605
.292	.1540	.3248	.2859	.2711	.2386	.2980	.2622
.294	.1550	.3270	.2878	.2729	.2402	.3000	.2640
.296	.1560	.3292	.2897	.2747	.2418	.3020	.2658
.298	.1571	.3313	.2916	.2765	.2434	.3040	.2675
.300	.1581	.3335	.2935	.2784	.2450	.3060	.2693

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.<sup>1</sup>

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
1.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
.9999	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07
.98	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13
.97	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20
.96	.27	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26
.95	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33
.94	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40
.93	.47	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46
.92	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53
.91	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60
90	.67	.66	.66	.66	.66	.66	.66	.66	.66	.66	.66	.66
.89	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73
.88	.80	.80	.80	.80	.80	.80	.79	.79	.79	.79	.79	.79
.87	.87	.87	.87	.87	.87	.87	.86	.86	.86	.86	.86	.86
.86	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93
.85	1.00	1.00	1.00	1.00	1.00	1.00	.99	.99	.99	.99	.99	.99
.84	.07	.07	.07	.07	.07	.07	1.06	1.06	1.06	1.06	1.06	1.06
.83	.14	.14	.14	.13	.13	.13	.13	.13	.13	.13	.13	.13
.82	.20	.20	.20	.20	.20	.20	.19	.19	.19	.19	.19	.19
.81	.27	.27	.27	.27	.27	.27	.26	.26	.26	.26	.26	.26
80	.34	.34	.34	.34	.34	.33	.33	.32	.32	.32	.32	.32
.79	.41	.41	.41	.40	.40	.40	.39	.39	.39	.39	.39	.39
.78	.48	.48	.48	.47	.47	.47	.46	.46	.46	.46	.46	.46
.77	.54	.54	.54	.54	.54	.53	.53	.53	.53	.53	.53	.53
.76	.61	.61	.61	.60	.60	.60	.59	.59	.59	.59	.59	.59
.75	.68	.68	.68	.67	.67	.67	.66	.66	.66	.66	.66	.66
.74	.75	.75	.75	.74	.74	.73	.73	.73	.72	.72	.72	.72
.73	.82	.81	.81	.81	.81	.80	.80	.80	.79	.79	.79	.79
.72	.88	.88	.88	.87	.87	.87	.86	.86	.86	.85	.85	.85
.71	.95	.95	.95	.94	.94	.94	.93	.93	.93	.92	.92	.92
70	2.02	2.02	2.02	2.01	2.01	2.01	2.00	2.00	2.00	.99	.99	.99
.69	.09	.09	.09	.08	.08	.08	.07	.07	.06	2.05	2.05	2.05
.68	.16	.15	.15	.14	.14	.14	.14	.14	.13	.12	.12	.12
.67	.23	.22	.22	.21	.21	.21	.20	.20	.20	.19	.19	.19
.66	.30	.29	.29	.28	.28	.28	.27	.27	.27	.26	.26	.26
.65	.37	.36	.36	.35	.35	.35	.34	.34	.33	.32	.32	.32
.64	.43	.43	.43	.42	.42	.42	.41	.41	.40	.39	.39	.39
.63	.50	.50	.50	.49	.49	.49	.48	.48	.47	.46	.46	.46
.62	.57	.57	.57	.56	.56	.56	.55	.54	.54	.53	.53	.53
.61	.64	.64	.64	.63	.63	.63	.62	.61	.60	.60	.59	.59
60	.71	.70	.70	.70	.70	.70	.69	.68	.67	.67	.66	.66
.59	.78	.77	.77	.77	.77	.77	.76	.75	.74	.74	.73	.73
.58	.85	.84	.84	.83	.83	.83	.82	.82	.81	.81	.80	.80
.57	.92	.91	.91	.90	.90	.90	.89	.88	.87	.87	.86	.86
.56	.99	.98	.98	.97	.97	.97	.96	.95	.94	.94	.93	.93
.55	3.06	3.05	3.05	3.04	3.04	3.04	3.03	3.02	3.01	3.01	3.00	3.00
.54	.13	.12	.12	.11	.11	.11	.10	.09	.08	.08	.07	.07
.53	.20	.19	.19	.18	.18	.18	.17	.16	.15	.15	.14	.14
.52	.27	.26	.26	.25	.25	.25	.24	.23	.22	.22	.21	.21
.51	.34	.33	.33	.32	.32	.32	.31	.30	.29	.28	.27	.27
50	.41	.40	.40	.39	.39	.39	.38	.37	.36	.35	.34	.34
.49	.49	.47	.47	.46	.46	.46	.45	.44	.43	.42	.41	.41
.48	.56	.54	.54	.53	.53	.53	.52	.51	.50	.49	.48	.48
.47	.63	.61	.61	.60	.60	.60	.59	.58	.57	.56	.55	.55
.46	.70	.68	.68	.67	.67	.67	.66	.65	.64	.63	.62	.62
.45	.77	.76	.75	.74	.74	.74	.73	.72	.70	.69	.68	.68
.44	.84	.83	.82	.81	.81	.81	.79	.78	.77	.76	.75	.75
.43	.91	.90	.89	.88	.88	.88	.86	.85	.84	.83	.82	.82
.42	.99	.97	.96	.95	.95	.95	.93	.92	.91	.90	.89	.89
.41	4.06	4.04	4.03	4.02	4.02	4.02	4.00	.99	.98	.97	.96	.96
40	.13	.11	.10	.10	.09	.09	.07	4.06	4.05	4.04	4.03	4.03
.39	.20	.18	.17	.17	.16	.16	.14	.13	.12	.11	.10	.10
.38	.28	.26	.25	.25	.24	.23	.21	.20	.19	.18	.17	.17
.37	.35	.33	.32	.32	.31	.30	.28	.27	.26	.25	.24	.24
.36	.42	.40	.39	.39	.38	.37	.36	.35	.33	.32	.31	.30
.35	.50	.48	.47	.46	.45	.44	.43	.42	.40	.39	.38	.37
.34	.57	.55	.54	.53	.52	.51	.50	.49	.47	.46	.45	.44
.33	.64	.62	.61	.60	.59	.58	.57	.56	.54	.53	.52	.51
.32	.71	.69	.68	.67	.66	.65	.64	.63	.61	.60	.59	.58
.31	.79	.77	.76	.75	.74	.73	.72	.70	.68	.67	.66	.65
30	.86	.84	.83	.82	.81	.80	.79	.77	.75	.74	.73	.72

<sup>1</sup> Compiled at the National Bureau of Standards. The table is based on data published in the Bulletin of the Bureau of Standards, Vol. 9, No. 3 (Reprint No. 197).

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 19

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9930	4.86	4.84	.83	.82	4.81	.80	.79	4.77	.75	.74	4.73	.72
29	.93	.91	.90	.89	.88	.87	.86	.84	.82	.81	.80	.79
28	5.01	.98	.97	.96	.95	.94	.93	.91	.89	.88	.87	.86
27	.08	5.06	5.04	5.03	5.02	5.01	5.00	.98	.96	.95	.94	.93
26	.16	.13	.12	.11	.10	.09	.07	5.05	5.03	5.02	5.01	5.00
25	.23	.21	.19	.18	.17	.16	.14	.12	.10	.09	.08	.07
24	.31	.28	.26	.25	.24	.23	.21	.20	.18	.16	.15	.14
23	.39	.36	.34	.33	.32	.31	.29	.27	.25	.23	.22	.21
22	.46	.43	.41	.40	.39	.38	.36	.34	.32	.30	.29	.28
21	.54	.51	.49	.48	.47	.46	.44	.42	.40	.38	.37	.36
20	.61	.58	.56	.55	.54	.53	.51	.49	.47	.45	.44	.43
19	.69	.66	.64	.62	.61	.60	.58	.56	.54	.52	.51	.50
18	.77	.73	.71	.70	.69	.68	.66	.64	.62	.59	.58	.57
17	.84	.81	.79	.77	.76	.75	.73	.71	.69	.66	.65	.64
16	.92	.88	.86	.85	.84	.83	.80	.78	.76	.74	.73	.72
15	.99	.96	.94	.92	.91	.90	.87	.85	.83	.81	.80	.79
14	6.07	6.03	6.01	6.00	.99	.98	.95	.93	.91	.88	.87	.86
13	.15	.11	.09	.07	6.06	6.05	6.02	6.00	.98	.95	.94	.93
12	.23	.18	.16	.15	.14	.13	.10	.08	6.05	6.02	6.01	6.00
11	.30	.26	.24	.22	.21	.20	.17	.15	.12	.10	.09	.08
10	.38	.34	.32	.30	.29	.28	.25	.23	.20	.17	.16	.15
09	.46	.41	.39	.37	.36	.35	.32	.30	.28	.25	.24	.23
08	.54	.49	.47	.45	.44	.43	.40	.38	.35	.32	.31	.30
07	.62	.57	.55	.53	.52	.51	.48	.45	.42	.39	.38	.37
06	.70	.65	.63	.60	.59	.58	.55	.53	.50	.47	.46	.45
05	.77	.73	.71	.68	.67	.66	.63	.60	.57	.54	.53	.52
04	.85	.80	.78	.75	.74	.73	.70	.68	.65	.62	.60	.59
03	.93	.88	.86	.83	.82	.81	.78	.75	.72	.69	.68	.67
02	7.01	.96	.93	.90	.89	.88	.85	.83	.80	.77	.75	.74
01	.09	7.04	7.01	.98	.97	.95	.92	.90	.87	.84	.82	.81
00	.17	.12	.09	7.06	7.05	7.03	7.00	.98	.94	.91	.90	.88
0.9899	.25	.19	.16	.13	.12	.10	.07	7.05	7.01	.98	.97	.95
98	.33	.27	.24	.21	.20	.18	.15	.13	.09	7.06	7.04	7.02
97	.41	.35	.32	.29	.28	.26	.23	.21	.17	.14	.12	.10
96	.50	.43	.40	.37	.36	.34	.31	.28	.24	.21	.19	.17
95	.58	.51	.48	.45	.44	.42	.39	.36	.32	.29	.27	.25
94	.66	.59	.56	.53	.52	.50	.47	.44	.40	.36	.34	.32
93	.74	.67	.64	.60	.59	.57	.54	.51	.47	.44	.42	.40
92	.82	.75	.72	.68	.67	.65	.62	.59	.55	.51	.49	.47
91	.90	.82	.79	.76	.75	.73	.70	.66	.62	.59	.57	.55
90	.98	.90	.87	.84	.83	.81	.78	.74	.70	.66	.64	.62
89	8.07	.98	.95	.92	.91	.89	.86	.82	.78	.74	.72	.70
88	.15	8.06	8.03	8.00	.98	.96	.93	.89	.85	.81	.79	.77
87	.23	.15	.11	.08	8.06	8.04	8.01	.97	.93	.89	.87	.85
86	.32	.23	.19	.16	.14	.12	.09	8.05	8.01	.96	.94	.92
85	.40	.31	.27	.24	.22	.20	.16	.12	.08	8.04	8.02	8.00
84	.48	.39	.35	.32	.30	.28	.24	.20	.16	.11	.09	.07
83	.57	.47	.43	.40	.38	.36	.32	.27	.23	.19	.17	.15
82	.65	.55	.51	.48	.46	.44	.40	.35	.31	.26	.24	.22
81	.73	.63	.59	.56	.54	.52	.48	.43	.39	.34	.32	.30
80	.82	.71	.67	.63	.61	.59	.55	.50	.46	.41	.39	.37
79	.90	.79	.75	.71	.69	.67	.63	.58	.54	.49	.47	.45
78	.98	.88	.84	.79	.77	.75	.71	.66	.61	.56	.54	.52
77	9.07	.96	.92	.87	.85	.83	.78	.73	.69	.64	.62	.60
76	.15	9.04	9.00	.95	.93	.91	.86	.81	.76	.71	.69	.67
75	.24	.13	.08	9.03	9.01	.99	.94	.89	.84	.79	.77	.75
74	.32	.21	.16	.11	.09	9.07	9.02	.96	.91	.86	.84	.82
73	.40	.29	.24	.19	.17	.15	.10	9.04	.99	.94	.92	.90
72	.49	.38	.33	.27	.25	.23	.18	.12	9.07	9.02	.97	.95
71	.57	.46	.41	.35	.33	.31	.26	.20	.15	.10	9.07	9.05
70	.66	.54	.49	.43	.41	.38	.33	.27	.22	.17	.14	.12
69	.74	.62	.57	.51	.49	.46	.41	.35	.30	.25	.22	.19
68	.82	.70	.65	.59	.57	.54	.49	.43	.37	.32	.29	.26
67	.91	.79	.74	.68	.65	.62	.57	.51	.45	.40	.37	.34
66	.99	.87	.82	.76	.73	.70	.65	.59	.53	.47	.44	.41
65	10.08	.95	.90	.84	.81	.78	.72	.66	.60	.54	.51	.48
64	.16	10.03	.98	.92	.89	.86	.80	.74	.68	.62	.59	.56
63	.25	.11	10.06	10.00	.97	.94	.88	.82	.76	.69	.66	.63
62	.33	.20	.14	.08	10.05	10.02	.96	.90	.84	.77	.74	.71
61	.42	.28	.22	.16	.13	.10	10.04	.98	.91	.84	.81	.78
60	.50	.36	.30	.24	.21	.18	.11	10.05	.99	.92	.89	.86

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 — 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9860	10.50	10.36	.30	.24	10.21	.18	.11	10.05	.99	.92	9.89	.86
59	.59	.44	.38	.32	.29	.26	.19	.13	10.06	.99	.96	.93
58	.68	.53	.47	.40	.37	.34	.27	.21	.14	10.07	10.04	10.00
57	.76	.61	.55	.48	.44	.41	.34	.28	.21	.14	.11	.07
56	.85	.69	.63	.56	.52	.49	.42	.36	.29	.22	.19	.15
55	.93	.78	.71	.64	.60	.57	.50	.44	.37	.30	.26	.23
54	11.02	.86	.79	.72	.68	.65	.58	.52	.45	.38	.34	.31
53	.11	.94	.87	.80	.76	.73	.66	.59	.52	.45	.41	.38
52	.19	11.03	.96	.88	.84	.81	.74	.67	.60	.53	.49	.45
51	.28	.11	11.04	.96	.92	.89	.82	.75	.67	.60	.56	.52
50	.37	.19	.12	11.04	11.00	.96	.89	.82	.74	.67	.63	.59
49	.46	.28	.20	.12	.08	11.04	.97	.90	.82	.75	.71	.67
48	.54	.36	.28	.20	.16	.12	11.05	.98	.90	.82	.78	.74
47	.63	.45	.36	.28	.24	.20	.13	11.05	.97	.90	.86	.82
46	.72	.53	.45	.37	.33	.29	.21	.13	11.05	.97	.93	.89
45	.81	.61	.53	.45	.41	.37	.29	.21	.13	11.05	11.01	.97
44	.89	.70	.62	.53	.49	.45	.37	.29	.21	.12	.08	11.04
43	.98	.78	.70	.61	.57	.53	.44	.36	.28	.20	.16	.12
42	12.07	.87	.78	.69	.65	.61	.52	.44	.36	.27	.23	.19
41	.16	.95	.86	.78	.73	.69	.60	.52	.44	.35	.31	.27
40	.25	12.04	.95	.86	.81	.77	.68	.60	.51	.42	.38	.34
39	.34	.12	12.03	.94	.89	.85	.76	.67	.58	.50	.46	.42
38	.43	.21	.12	12.03	.98	.93	.84	.75	.66	.57	.53	.49
37	.52	.29	.20	.11	12.06	12.01	.92	.83	.74	.65	.61	.57
36	.61	.38	.28	.19	.14	.09	12.00	.91	.82	.73	.68	.64
35	.70	.47	.37	.27	.22	.17	.07	.98	.89	.80	.76	.72
34	.79	.55	.45	.35	.30	.25	.15	12.06	.97	.88	.83	.79
33	.88	.64	.54	.44	.39	.34	.24	.14	12.05	.96	.91	.86
32	.97	.73	.63	.52	.47	.42	.32	.22	.12	12.03	.98	.93
31	13.06	.81	.71	.60	.55	.50	.40	.30	.20	.11	12.06	12.01
30	.16	.90	.79	.68	.63	.58	.48	.38	.28	.19	.14	.09
29	.25	.99	.88	.77	.71	.66	.56	.46	.36	.26	.21	.16
28	.34	13.07	.96	.85	.80	.74	.64	.54	.44	.34	.29	.24
27	.43	.16	13.05	.93	.88	.82	.72	.62	.52	.42	.37	.32
26	.52	.25	.13	13.01	.96	.90	.80	.70	.59	.49	.44	.39
25	.61	.34	.22	.10	13.04	.99	.88	.78	.67	.57	.52	.47
24	.71	.43	.31	.19	.13	13.08	.97	.86	.75	.65	.60	.55
23	.80	.51	.39	.27	.21	.16	13.05	.94	.83	.72	.67	.62
22	.89	.60	.47	.35	.29	.24	.13	13.02	.91	.80	.75	.70
21	.98	.68	.56	.44	.38	.33	.22	.10	.99	.88	.82	.77
20	14.08	.77	.64	.52	.46	.40	.29	.18	13.06	.95	.90	.85
19	.17	.86	.73	.61	.55	.49	.37	.26	.15	13.04	.98	.93
18	.26	.95	.82	.69	.63	.57	.45	.34	.22	.11	13.05	13.00
17	.36	14.04	.91	.78	.72	.66	.54	.42	.30	.19	.13	.08
16	.45	.13	14.00	.87	.80	.74	.62	.50	.38	.27	.21	.16
15	.55	.22	.08	.95	.88	.82	.70	.58	.46	.34	.28	.23
14	.64	.30	.17	14.04	.97	.91	.78	.66	.54	.42	.36	.30
13	.74	.39	.25	.12	14.05	.99	.86	.74	.62	.50	.44	.38
12	.83	.48	.34	.20	.13	14.07	.94	.82	.70	.58	.52	.46
11	.92	.57	.43	.29	.22	.16	14.03	.90	.77	.65	.59	.53
10	15.02	.66	.51	.37	.30	.24	.11	.98	.85	.73	.67	.61
09	.11	.75	.60	.46	.39	.32	.19	14.06	.93	.81	.75	.69
08	.21	.84	.79	.54	.47	.40	.27	.14	14.01	.88	.82	.76
07	.30	.93	.67	.62	.55	.48	.35	.22	.09	.96	.90	.84
06	.40	15.02	.76	.71	.64	.57	.43	.30	.17	14.04	.98	.92
05	.49	.11	.95	.79	.72	.65	.51	.38	.25	.12	14.05	.99
04	.58	.20	15.04	.88	.81	.74	.60	.46	.33	.20	.13	14.07
03	.67	.28	.12	.96	.89	.82	.68	.54	.41	.28	.21	.15
02	.77	.37	.21	15.05	.97	.90	.76	.62	.49	.36	.29	.23
01	.87	.46	.30	.14	15.06	.99	.84	.70	.56	.43	.36	.30
00	.96	.55	.39	.23	.15	15.07	.92	.78	.64	.51	.44	.38
0.9799	16.06	.44	.32	.24	.16	.16	15.01	.86	.72	.59	.52	.46
98	.15	.73	.46	.40	.32	.24	.09	.94	.80	.67	.60	.54
97	.25	.82	.55	.49	.41	.33	.17	15.02	.88	.74	.67	.61
96	.35	.91	.64	.57	.49	.41	.26	.11	.96	.82	.75	.68
95	.44	16.00	.83	.66	.58	.50	.34	.19	15.04	.90	.83	.76
94	.54	.10	.92	.75	.66	.59	.43	.27	.12	.98	.91	.84
93	.63	.19	16.01	.84	.75	.67	.51	.35	.20	15.05	.98	.91
92	.73	.28	.10	.93	.84	.76	.59	.43	.28	.13	15.06	.99
91	.83	.37	.19	16.01	.92	.84	.67	.51	.36	.21	.14	15.07
90	.92	.46	.27	.09	16.00	.92	.75	.59	.44	.29	.22	.15

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent 19  
specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
	15.56											
0.9790	16.92	16.46	16.27	16.09	16.00	15.92	15.75	15.59	15.44	15.29	15.22	15.15
89	17.02	.55	.26	.18	.09	16.01	.84	.67	.52	.37	.30	.23
88	.12	.64	.45	.27	.18	.10	.93	.76	.61	.45	.38	.31
87	.22	.73	.54	.36	.27	.18	16.01	.84	.68	.52	.45	.38
86	.32	.83	.63	.44	.35	.26	.09	.92	.76	.60	.53	.46
85	.42	.92	.72	.53	.44	.35	.17	16.00	.84	.68	.61	.53
84	.51	17.01	.81	.62	.53	.44	.26	.08	.92	.76	.69	.61
83	.61	.10	.90	.70	.61	.52	.34	.17	.10	.84	.77	.69
82	.71	.20	.99	.79	.70	.61	.43	.25	16.08	.92	.84	.76
81	.81	.29	17.08	.88	.78	.69	.51	.33	.16	16.00	.92	.84
80	.91	.38	.17	.97	.87	.78	.59	.41	.24	.08	16.00	.92
79	18.01	.47	.26	17.06	.96	.87	.68	.50	.33	.16	.08	16.00
78	.11	.57	.35	.14	17.04	.95	.76	.58	.41	.24	.16	.08
77	.21	.66	.44	.23	.13	17.04	.85	.66	.49	.32	.24	.16
76	.31	.75	.53	.32	.22	.12	.93	.74	.57	.40	.32	.24
75	.41	.84	.62	.40	.30	.20	17.01	.83	.65	.48	.40	.32
74	.51	.94	.72	.50	.39	.29	.10	.91	.73	.56	.48	.40
73	.61	18.03	.81	.59	.48	.38	.18	.99	.81	.64	.56	.48
72	.71	.12	.90	.68	.57	.47	.27	17.07	.89	.72	.63	.55
71	.81	.22	.99	.76	.65	.55	.35	.16	.97	.80	.71	.63
70	.91	.31	18.08	.85	.74	.63	.43	.24	17.05	.88	.79	.71
69	19.01	.40	.16	.94	.83	.72	.52	.32	.14	.96	.87	.79
68	.11	.50	.25	18.02	.91	.80	.60	.40	.22	17.04	.95	.86
67	.21	.59	.34	.11	18.00	.89	.69	.49	.30	.12	17.03	.94
66	.32	.69	.44	.20	.09	.98	.78	.57	.38	.20	.11	17.02
65	.42	.78	.53	.29	.18	18.07	.86	.65	.46	.28	.19	.10
64	.52	.88	.63	.38	.27	.16	.95	.74	.55	.36	.27	.17
63	.62	.97	.71	.47	.35	.24	18.03	.82	.62	.43	.35	.25
62	.72	19.07	.81	.56	.44	.33	.11	.90	.70	.51	.43	.33
61	.83	.16	.80	.65	.53	.42	.20	.98	.78	.59	.50	.41
60	.93	.26	.99	.74	.62	.50	.28	18.07	.87	.67	.58	.49
59	20.03	.35	19.08	.83	.71	.60	.37	.15	.95	.75	.66	.56
58	.13	.45	.18	.92	.80	.69	.46	.23	18.03	.83	.74	.64
57	.23	.54	.27	19.01	.88	.77	.54	.32	.11	.91	.82	.72
56	.33	.64	.36	.10	.97	.86	.62	.40	.19	.99	.90	.80
55	.43	.73	.45	.19	19.06	.94	.70	.48	.27	18.07	.98	.88
54	.53	.83	.55	.28	.15	19.03	.79	.57	.36	.15	18.06	.96
53	.63	.92	.64	.37	.24	.12	.88	.65	.44	.23	.13	18.04
52	.73	20.02	.73	.46	.33	.21	.96	.73	.52	.31	.21	.12
51	.83	.11	.82	.55	.42	.30	19.05	.82	.60	.39	.29	.19
50	.93	.20	.91	.64	.50	.38	.13	.90	.68	.47	.37	.27
49	21.03	.30	20.01	.73	.59	.47	.22	.98	.76	.55	.45	.35
48	.13	.39	.10	.82	.68	.56	.31	19.07	.85	.64	.53	.43
47	.23	.48	.19	.91	.77	.65	.39	.15	.93	.72	.61	.51
46	.33	.58	.28	20.00	.86	.74	.48	.24	19.01	.80	.69	.59
45	.43	.67	.37	.09	.95	.82	.56	.32	.09	.88	.77	.67
44	.52	.76	.46	.17	20.03	.90	.64	.40	.17	.96	.85	.75
43	.62	.86	.55	.26	.12	.99	.73	.49	.26	19.04	.93	.83
42	.72	.95	.64	.35	.21	20.08	.82	.57	.34	.12	19.01	.91
41	.82	21.04	.73	.44	.30	.17	.91	.66	.42	.20	.09	.98
40	.92	.14	.82	.53	.38	.25	.99	.74	.50	.28	.17	19.06
39	22.02	.23	.91	.62	.47	.34	20.07	.82	.58	.35	.24	.23
38	.12	.32	21.00	.71	.56	.43	.16	.90	.66	.43	.32	.31
37	.22	.41	.09	.79	.64	.51	.24	.98	.74	.51	.40	.29
36	.31	.50	.18	.88	.73	.59	.32	20.06	.82	.59	.48	.37
35	.41	.60	.27	.97	.82	.68	.41	.15	.90	.67	.56	.45
34	.51	.69	.36	21.05	.90	.77	.50	.24	.99	.75	.64	.53
33	.61	.78	.45	.14	.99	.85	.58	.32	20.07	.83	.72	.61
32	.71	.87	.54	.23	21.08	.94	.66	.40	.15	.91	.80	.68
31	.80	.96	.63	.32	.16	21.02	.74	.48	.23	.99	.87	.76
30	.90	22.05	.72	.41	.25	.11	.83	.56	.31	20.07	.95	.84
29	23.00	.14	.81	.50	.34	.20	.91	.64	.39	.15	20.03	.92
28	.10	.24	.90	.58	.42	.28	.99	.72	.47	.23	.11	20.00
27	.19	.33	.99	.67	.51	.36	21.07	.80	.55	.31	.19	.08
26	.29	.42	22.08	.76	.59	.45	.16	.89	.63	.39	.27	.16
25	.38	.51	.17	.84	.68	.53	.24	.97	.71	.46	.34	.23
24	.48	.60	.26	.93	.77	.62	.33	21.05	.79	.54	.42	.30
23	.58	.69	.34	22.01	.85	.70	.41	.13	.87	.62	.50	.38
22	.67	.78	.43	.10	.94	.78	.49	.21	.95	.70	.58	.46
21	.77	.87	.52	.19	22.03	.87	.58	.30	21.03	.78	.66	.54
20	.87	.96	.61	.27	.11	.96	.66	.38	.11	.86	.73	.61

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9720	23.87	22.96	.61	.27	22.11	.96	.66	21.38	.11	.86	20.73	.61
19	.96	23.06	.70	.36	.19	22.04	.74	.46	.19	.94	.81	.69
18	24.06	.15	.79	.45	.28	.12	.82	.54	.27	21.02	.89	.77
17	.15	.24	.88	.54	.36	.21	.91	.62	.35	.10	.97	.85
16	.25	.33	.96	.62	.45	.30	.99	.70	.43	.17	21.05	.92
15	.34	.42	23.05	.70	.53	.38	22.08	.79	.51	.24	.12	.99
14	.43	.51	.14	.79	.62	.46	.16	.87	.59	.33	20	21.08
13	.53	.60	.22	.87	.70	.54	.24	.95	.67	.40	.27	.15
12	.62	.69	.31	.96	.79	.63	.32	22.03	.75	.88	.35	.22
11	.72	.78	.40	23.04	.87	.71	.40	.11	.83	.56	.43	.30
10	.81	.87	.49	.13	.96	.80	.49	.19	.91	.64	.50	.37
09	.91	.95	.57	.21	23.04	.88	.57	.27	.99	.72	.58	.45
08	25.00	24.04	.66	.30	.13	.97	.65	.35	22.07	.80	.66	.53
07	.09	.13	.74	.38	.21	23.05	.73	.43	.14	.87	.73	.60
06	.19	.22	.83	.47	.29	.13	.81	.51	.22	.95	.81	.68
05	.28	.31	.92	.56	.38	.22	.90	.59	.30	22.03	.89	.76
04	.38	.40	24.00	.64	.46	.30	.98	.67	.33	.10	.96	.83
03	.47	.49	.09	.73	.55	.38	23.06	.75	.46	.18	22.04	.91
02	.57	.58	.18	.81	.63	.46	.14	.83	.53	.25	.11	.98
01	.66	.66	.26	.89	.71	.54	.21	.90	.61	.33	.19	22.06
00	.75	.75	.35	.98	.80	.63	.30	.98	.69	.41	.27	.14
0.9699	.85	.84	.44	24.06	.88	.72	.38	23.06	.77	.48	.34	.21
98	.94	.93	.53	.15	.97	.80	.46	.14	.84	.55	.42	.28
97	26.04	25.01	.61	.23	24.05	.88	.54	.22	.92	.63	.49	.35
96	.13	.10	.69	.31	.13	.96	.62	.30	23.00	.71	.57	.43
95	.22	.19	.78	.40	.22	24.05	.70	.38	.08	.78	.64	.50
94	.31	.28	.86	.48	.30	.13	.78	.45	.15	.86	.72	.58
93	.41	.36	.95	.57	.38	.21	.86	.53	.23	.94	.80	.66
92	.50	.45	25.04	.65	.47	.29	.94	.61	.31	23.01	.87	.74
91	.59	.54	.13	.74	.55	.37	24.02	.69	.38	.08	.95	.81
90	.69	.62	.21	.82	.63	.45	.10	.77	.46	.16	23.02	.88
89	.78	.71	.29	.90	.72	.53	.18	.84	.53	.23	.10	.96
88	.87	.80	.38	.98	.80	.61	.26	.92	.61	.31	.17	23.03
87	.96	.89	.46	25.07	.88	.69	.34	24.00	.68	.38	.24	.10
86	27.05	.98	.55	.15	.97	.77	.42	.68	.76	.46	.32	.18
85	.15	26.06	.63	.23	25.05	.85	.50	.16	.84	.53	.39	.25
84	.24	.15	.72	.32	.13	.94	.58	.23	.92	.61	.47	.33
83	.33	.24	.80	.40	.21	25.02	.66	.31	.99	.68	.54	.40
82	.42	.33	.89	.48	.29	.10	.74	.39	24.06	.75	.61	.47
81	.51	.41	.97	.57	.37	.18	.81	.47	.14	.83	.69	.54
80	.60	.50	26.06	.65	.45	.26	.89	.54	.21	.90	.76	.61
79	.69	.59	.14	.73	.53	.34	.97	.62	.30	.98	.84	.69
78	.78	.67	.22	.81	.61	.42	25.05	.70	.37	24.06	.91	.77
77	.87	.76	.31	.89	.69	.50	.13	.78	.45	.14	.99	.84
76	.96	.84	.39	.97	.77	.58	.21	.85	.52	.21	24.06	.91
75	28.05	.93	.47	26.05	.85	.66	.29	.93	.60	.29	.13	.99
74	.14	27.01	.56	.14	.94	.74	.37	25.01	.68	.36	.21	24.06
73	.23	.10	.64	.22	26.02	.82	.45	.09	.75	.43	.28	.13
72	.32	.19	.73	.30	.10	.90	.53	.16	.83	.51	.36	.20
71	.41	.27	.81	.38	.18	.98	.60	.24	.90	.58	.43	.28
70	.50	.36	.89	.46	.26	26.06	.68	.32	.98	.66	.50	.35
69	.59	.44	.97	.54	.34	.14	.76	.40	25.06	.73	.58	.42
68	.68	.52	27.05	.63	.42	.22	.84	.47	.13	.81	.65	.50
67	.77	.61	.14	.71	.50	.30	.92	.55	.20	.88	.73	.57
66	.86	.69	.22	.79	.58	.38	.99	.63	.28	.95	.80	.64
65	.95	.77	.30	.87	.66	.46	26.07	.70	.36	25.03	.87	.72
64	29.04	.86	.39	.95	.74	.54	.15	.78	.44	.11	.95	.79
63	.12	.94	.47	27.03	.82	.62	.23	.86	.51	.18	25.02	.86
62	.21	28.02	.55	.11	.90	.70	.31	.94	.59	.25	.09	.93
61	.30	.11	.64	.19	.98	.77	.38	26.02	.66	.33	.17	25.01
60	.39	.19	.72	.27	27.06	.85	.46	.09	.74	.40	.24	.08
59	.47	.28	.81	.35	.13	.93	.54	.17	.82	.48	.31	.15
58	.56	.36	.89	.43	.21	27.01	.61	.24	.89	.56	.39	.23
57	.65	.44	.97	.51	.29	.09	.69	.32	.97	.63	.46	.30
56	.74	.53	27.05	.59	.37	.17	.77	.39	26.04	.70	.53	.37
55	.82	.61	.13	.67	.45	.25	.85	.47	.11	.77	.61	.45
54	.91	.69	.21	.75	.53	.33	.93	.55	.19	.85	.68	.52
53	30.00	.78	.29	.83	.61	.41	27.00	.62	.26	.92	.75	.59
52	.09	.86	.37	.91	.69	.49	.08	.70	.34	.99	.82	.66
51	.17	.94	.45	.99	.77	.56	.16	.78	.41	26.06	.90	.74
50	.26	29.03	.53	28.07	.85	.64	.23	.85	.49	.14	.97	.81



Percentages by volume at 60°F of ethyl alcohol corresponding to apparent 19  
specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9650	30.26	29.03	.53	.07	27.85	.64	.23	26.85	.49	.14	25.97	.81
49	.34	.11	.61	.15	.93	.72	.31	.92	.56	.21	26.04	.89
48	.43	.19	.69	.23	28.01	.79	.38	27.00	.64	.29	.11	.96
47	.52	.27	.73	.31	.09	.87	.46	.07	.71	.36	.19	26.03
46	.60	.35	.85	.39	.16	.95	.53	.15	.78	.43	.26	.10
45	.69	.44	.93	.47	.24	28.03	.61	.22	.85	.51	.33	.17
44	.78	.52	29.02	.55	.32	.10	.69	.30	.93	.58	.40	.24
43	.86	.60	.10	.63	.40	.18	.76	.37	27.00	.65	.47	.31
42	.95	.68	.18	.71	.47	.26	.84	.44	.07	.72	.54	.38
41	31.03	.76	.26	.79	.55	.34	.91	.52	.14	.79	.61	.45
40	.11	.85	.34	.86	.63	.41	.99	.59	.22	.86	.69	.52
39	.20	.93	.42	.93	.71	.49	28.06	.67	.29	.93	.76	.59
38	.28	30.01	.50	29.01	.78	.56	.14	.74	.37	27.01	.83	.66
37	.36	.09	.58	.09	.86	.64	.21	.81	.44	.08	.90	.73
36	.44	.17	.66	.17	.94	.72	.29	.89	.51	.15	.97	.80
35	.52	.25	.74	.25	29.02	.80	.37	.96	.58	.22	27.04	.87
34	.61	.34	.82	.33	.09	.87	.44	28.04	.66	.29	.11	.94
33	.69	.42	.90	.41	.17	.95	.52	.11	.73	.36	.18	27.01
32	.77	.50	.98	.49	.25	29.03	.60	.19	.80	.43	.25	.08
31	.85	.58	30.06	.57	.33	.11	.67	.26	.87	.50	.32	.15
30	.93	.66	.13	.64	.40	.18	.74	.33	.95	.58	.39	.22
29	32.02	.74	.21	.72	.48	.26	.82	.41	28.02	.65	.46	.29
28	.09	.82	.29	.79	.56	.33	.89	.48	.10	.72	.54	.36
27	.17	.89	.36	.87	.64	.41	.97	.56	.17	.79	.61	.43
26	.25	.97	.44	.95	.71	.48	29.04	.63	.24	.86	.68	.50
25	.33	31.05	.52	30.03	.79	.56	.12	.70	.31	.93	.75	.57
24	.41	.13	.60	.10	.87	.64	.20	.78	.38	28.00	.82	.64
23	.49	.20	.67	.17	.95	.71	.27	.85	.45	.07	.89	.71
22	.57	.28	.75	.25	30.02	.79	.35	.93	.52	.14	.96	.78
21	.65	.36	.83	.33	.10	.86	.42	29.00	.59	.21	28.03	.85
20	.72	.44	.91	.41	.17	.94	.50	.07	.67	.29	.10	.92
19	.80	.52		.25	.25	30.01	.57	.14	.74	.36	.17	.99
18	.88	.59		.32	.09	.65	.22	.82	.43	.24	28.06	
17	.96	.67		.40	.16	.72	.29	.89	.50	.31	.13	
16	33.04	.75		.47	.24	.79	.36	.96	.57	.38	.20	
15	.12	.82		.54	.31	.86	.43	29.03	.64	.45	.27	
14	.19	.90		.62	.39	.94	.51	.10	.71	.52	.34	
13	.27	.98		.69	.46	30.01	.58	.17	.78	.59	.41	
12	.35	32.05		.77	.53	.08	.65	.24	.85	.66	.48	
11	.43	.13		.84	.61	.15	.72	.31	.92	.73	.55	
10	.50	.21			.92	.68	.23	.80	.39	.99	.80	.62
09	.58	.28			.99	.75	.30	.87	.46	29.06	.87	.69
08	.66	.36		31.07	.83	.38	.94	.53	.13	.94	.76	
07	.74	.43		.14	.90	.45	30.01	.60	.20	29.01	.83	
06	.81	.51		.21	.98	.52	.09	.67	.27	.08	.90	
05	.89	.58		.29	31.05	.59	.16	.74	.34	.15	.97	
04	.97	.66		.36	.13	.66	.23	.81	.41	.22	29.04	
03	34.05	.73		.43	.20	.73	.30	.88	.48	.29	.11	
02	.12	.81		.51	.28	.80	.37	.95	.55	.36	.18	
01	.20	.88		.58	.35	.88	.44	30.02	.62	.43	.25	
00	.27	.96			.65	.42	.95	.51	.09	.69	.50	.31
0.9599	.35	33.03			.73			.58	.16	.76	.57	.38
98	.42	.10			.80			.65	.23	.83	.63	.45
97	.50	.18			.87			.72	.30	.90	.70	.51
96	.57	.25			.95			.79	.37	.97	.77	.58
95	.65	.32		32.02				.87	.44	30.04	.84	.65
94	.72	.40			.09			.94	.51	.11	.91	.72
93	.80	.47			.16			31.01	.68	.18	.98	.79
92	.87	.54			.23			.08	.65	.25	30.05	.86
91	.95	.62			.30			.15	.72	.32	.12	.93
90	35.02	.69			.37			.22	.79	.38	.18	.99
89	.09	.76			.44			.28		.25	30.06	
88	.17	.84			.51			.35		.32	.13	
87	.24	.91			.58			.42		.39	.20	
86	.31	.98			.65			.49		.46	.27	
85	.38	34.05			.73			.56		.52	.33	
84	.46	.12			.80			.63		.59	.40	
83	.53	.20			.87			.70		.66	.47	
82	.60	.27			.94			.77		.73	.54	
81	.67	.34		33.01				.84		.80	.61	
80	.75	.41			.08			.91		.86	.67	

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9580	35.75	34.41	33.08	31.91	30.86	0.9510	40.46	39.10	37.71	36.47	35.34
79	.82	.48	.15	.98	.93	09	.52	.16	.78	.53	.40
78	.89	.56	.22	32.05	31.00	08	.58	.23	.84	.59	.46
77	.96	.63	.29	.11	.07	07	.65	.29	.90	.65	.52
76	36.04	.70	.36	.18	.13	06	.71	.35	.96	.72	.58
75	.11	.77	.43	.25	.20	05	.77	.41	38.02	.78	.64
74	.18	.84	.50	.32	.26	04	.84	.48	.09	.84	.71
73	.25	.91	.57	.38	.33	03	.90	.54	.15	.90	.77
72	.32	.98	.64	.45	.39	02	.96	.60	.21	.96	.83
71	.39	35.05	.71	.52	.46	01	41.02	.67	.27	37.02	.89
70	.46	.12	.78	.58	.53	00	.09	.73	.33	.09	.95
69	.53	.19	.85	.65	.59	0.9499	.15	.79	.40	.15	36.01
68	.60	.26	.92	.72	.66	98	.21	.85	.46	.21	.07
67	.67	.33	.99	.79	.72	97	.27	.91	.52	.27	.13
66	.74	.40	34.05	.85	.79	96	.33	.98	.58	.33	.19
65	.81	.47	.12	.92	.86	95	.40	40.04	.64	.39	.25
64	.88	.54	.19	.99	.92	94	.46	.10	.70	.45	.31
63	.95	.61	.26	33.05	.99	93	.52	.16	.77	.51	.37
62	37.02	.68	.32	.12	32.05	92	.58	.22	.83	.57	.43
61	.09	.75	.39	.19	.12	91	.64	.29	.89	.63	.49
60	.16	.82	.46	.25	.18	90	.70	.35	.95	.70	.55
59	.22	.88	.53	.32	.25	89	.77	.41	39.01	.76	.61
58	.29	.95	.59	.39	.31	88	.83	.47	.07	.82	.67
57	.36	36.02	.66	.45	.37	87	.89	.53	.13	.88	.73
56	.43	.09	.73	.52	.44	86	.95	.59	.20	.94	.79
55	.50	.15	.80	.59	.50	85	42.01	.65	.26	38.00	.85
54	.56	.22	.86	.65	.57	84	.07	.71	.32	.06	.91
53	.63	.29	.93	.72	.63	83	.13	.78	.38	.12	.97
52	.70	.36	35.00	.79	.70	82	.19	.84	.44	.18	37.03
51	.77	.42	.07	.85	.76	81	.25	.90	.50	.24	.09
50	.84	.49	.13	.92	.83	80	.31	.96	.56	.30	.15
49	.90	.56	.20	.99	.89	79	.37	41.02	.62	.36	.21
48	.97	.63	.26	34.05	.95	78	.43	.08	.68	.42	.26
47	38.04	.69	.33	.12	33.02	77	.49	.14	.74	.48	.32
46	.11	.76	.39	.18	.08	76	.55	.20	.80	.54	.38
45	.17	.83	.46	.25	.15	75	.61	.26	.87	.60	.44
44	.24	.89	.53	.31	.21	74	.67	.32	.93	.66	.50
43	.31	.96	.59	.38	.27	73	.73	.38	.99	.72	.56
42	.37	37.03	.66	.44	.34	72	.80	.44	40.05	.78	.62
41	.44	.09	.72	.51	.40	71	.86	.50	.11	.84	.68
40	.51	.16	.79	.57	.46	70	.92	.56	.17	.90	.74
39	.57	.23	.86	.64	.53	69	.98	.62	.22	.96	.79
38	.64	.29	.92	.70	.59	68	43.04	.68	.28	39.02	.85
37	.71	.36	.99	.77	.66	67	.09	.74	.34	.08	.91
36	.77	.42	36.05	.83	.72	66	.15	.80	.40	.13	.97
35	.84	.49	.12	.90	.78	65	.21	.86	.46	.19	38.03
34	.91	.56	.18	.96	.85	64	.27	.92	.52	.25	.09
33	.97	.62	.25	35.03	.91	63	.33	.98	.58	.31	.15
32	39.04	.69	.31	.09	.97	62	.39	42.04	.64	.37	.20
31	.10	.75	.38	.15	34.04	61	.45	.09	.70	.43	.26
30	.17	.82	.44	.22	.10	60	.51	.15	.76	.49	.32
29	.23	.88	.51	.28	.16	59	.57	.21	.82	.54	.38
28	.30	.95	.57	.34	.22	58	.63	.27	.88	.60	.44
27	.36	38.01	.64	.41	.29	57	.69	.33	.93	.66	.49
26	.43	.07	.70	.47	.35	56	.75	.39	.99	.72	.55
25	.49	.14	.77	.53	.41	55	.80	.45	41.05	.78	.61
24	.56	.20	.83	.59	.47	54	.86	.51	.11	.84	.67
23	.62	.27	.90	.66	.53	53	.92	.57	.17	.89	.73
22	.69	.33	.96	.72	.60	52	.98	.63	.23	.95	.78
21	.75	.39	37.02	.78	.66	51	44.04	.69	.28	40.01	.84
20	.82	.46	.09	.85	.72	50	.10	.74	.34	.07	.90
19	.88	.52	.15	.91	.78	49	.16	.80	.40	.13	.96
18	.95	.59	.21	.97	.84	48	.21	.86	.46	.18	39.02
17	40.01	.65	.28	36.04	.91	47	.27	.92	.51	.24	.07
16	.08	.72	.34	.10	.97	46	.33	.98	.57	.30	.13
15	.14	.78	.40	.16	35.04	45	.39	43.04	.63	.35	.19
14	.20	.84	.46	.22	.10	44	.45	.09	.69	.41	.24
13	.27	.91	.52	.28	.16	43	.50	.15	.75	.47	.30
12	.33	.97	.59	.35	.22	42	.56	.21	.80	.53	.36
11	.39	39.04	.65	.41	.28	41	.62	.27	.86	.58	.41
10	.46	.10	.71	.47	.34	40	.68	.33	.92	.64	.47

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 19

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56						15.56				
0.9440	44.68	43.33	41.92	40.64	39.47	0.9370	48.53	47.20	45.81	44.52	43.33
39	.73	.39	.98	.70	.53	69	.58	.26	.86	.58	.38
38	.79	.44	42.03	.75	.59	68	.63	.31	.91	.63	.43
37	.85	.50	.09	.81	.64	67	.69	.36	.97	.68	.49
36	.91	.56	.15	.87	.70	66	.74	.42	46.02	.74	.54
35	.97	.62	.21	.93	.76	65	.79	.47	.07	.79	.59
34	45.02	.67	.26	.98	.81	64	.85	.52	.13	.84	.65
33	.08	.73	.32	41.04	.87	63	.90	.58	.18	.90	.70
32	.14	.78	.38	.10	.93	62	.95	.63	.23	.95	.75
31	.19	.85	.43	.15	.98	61	49.01	.68	.29	45.01	.81
30	.25	.90	.49	.21	40.04	60	.06	.73	.34	.06	.86
29	.31	.96	.55	.27	.09	59	.11	.79	.39	.11	.91
28	.36	44.02	.61	.32	.15	58	.16	.84	.45	.16	.97
27	.42	.07	.66	.38	.21	57	.21	.89	.50	.22	44.02
26	.47	.13	.72	.44	.26	56	.26	.94	.55	.27	.07
25	.53	.18	.78	.49	.32	55	.32	48.00	.61	.32	.13
24	.59	.24	.83	.55	.37	54	.37	.05	.66	.37	.18
23	.64	.30	.89	.60	.43	53	.42	.10	.71	.43	.23
22	.70	.35	.95	.66	.48	52	.47	.15	.77	.48	.28
21	.76	.41	43.01	.72	.54	51	.52	.21	.82	.53	.34
20	.81	.46	.06	.77	.59	50	.58	.26	.87	.58	.39
19	.87	.52	.12	.83	.65	49	.63	.31	.93	.64	.44
18	.93	.58	.17	.89	.71	48	.68	.36	.98	.69	.49
17	.98	.63	.23	.94	.76	47	.73	.41	47.03	.74	.54
16	46.04	.69	.29	42.00	.82	46	.78	.47	.08	.79	.60
15	.09	.74	.34	.06	.87	45	.83	.52	.14	.85	.65
14	.15	.80	.40	.11	.93	44	.89	.57	.19	.90	.70
13	.20	.86	.46	.17	.98	43	.94	.62	.24	.95	.75
12	.26	.91	.51	.22	41.04	42	.99	.68	.29	46.01	.81
11	.31	.97	.57	.28	.09	41	50.04	.73	.34	.06	.86
10	.37	45.03	.62	.33	.15	40	.09	.78	.40	.11	.91
09	.43	.08	.68	.39	.20	39	.14	.83	.45	.16	.96
08	.48	.14	.74	.44	.26	38	.19	.88	.50	.21	45.02
07	.54	.19	.79	.50	.31	37	.24	.94	.55	.27	.07
06	.59	.25	.85	.56	.37	36	.30	.99	.60	.32	.12
05	.65	.30	.90	.61	.42	35	.35	49.04	.66	.37	.17
04	.70	.36	.96	.67	.48	34	.40	.09	.71	.42	.22
03	.76	.42	44.02	.72	.53	33	.45	.14	.76	.47	.27
02	.81	.47	.07	.78	.59	32	.50	.19	.81	.53	.33
01	.87	.53	.13	.83	.64	31	.55	.25	.86	.58	.38
00	.92	.58	.18	.89	.70	30	.60	.30	.92	.63	.43
0.9399	.98	.64	.23	.94	.75	29	.65	.35	.97	.68	.48
98	47.03	.69	.29	43.00	.81	28	.70	.40	48.02	.73	.53
97	.09	.74	.34	.05	.86	27	.75	.45	.07	.79	.59
96	.14	.80	.40	.11	.92	26	.81	.50	.12	.84	.64
95	.19	.85	.45	.16	.97	25	.86	.55	.17	.89	.69
94	.25	.91	.51	.22	42.03	24	.91	.60	.22	.94	.74
93	.30	.96	.56	.27	.08	23	.96	.65	.28	.99	.79
92	.35	46.01	.62	.33	.14	22	51.01	.70	.33	47.05	.84
91	.41	.07	.67	.38	.19	21	.06	.75	.38	.10	.90
90	.46	.12	.73	.44	.24	20	.11	.80	.43	.15	.95
89	.52	.18	.78	.49	.30	19	.16	.85	.48	.20	46.00
88	.57	.23	.84	.55	.35	18	.21	.90	.53	.25	.05
87	.62	.29	.89	.60	.41	17	.26	.95	.58	.30	.10
86	.68	.34	.95	.66	.46	16	.31	50.00	.63	.35	.15
85	.73	.39	45.00	.71	.52	15	.36	.05	.68	.40	.20
84	.78	.45	.05	.77	.57	14	.41	.10	.73	.45	.26
83	.84	.50	.11	.82	.63	13	.46	.16	.79	.50	.31
82	.89	.56	.16	.87	.68	12	.51	.21	.84	.55	.36
81	.95	.61	.22	.93	.73	11	.56	.26	.89	.60	.41
80	48.00	.67	.27	.98	.79	10	.61	.31	.94	.65	.46
79	.05	.72	.32	44.04	.84	09	.66	.36	.99	.71	.51
78	.11	.77	.38	.09	.90	08	.71	.41	49.04	.76	.56
77	.16	.83	.43	.15	.95	07	.76	.46	.09	.81	.61
76	.21	.88	.48	.20	43.01	06	.81	.51	.14	.86	.66
75	.26	.94	.54	.25	.06	05	.86	.56	.19	.91	.71
74	.32	.99	.59	.31	.11	04	.91	.61	.24	.96	.77
73	.37	47.04	.65	.36	.17	03	.96	.66	.29	48.01	.82
72	.42	.10	.70	.41	.22	02	52.01	.71	.34	.06	.87
71	.48	.15	.75	.47	.27	01	.06	.76	.39	.11	.92
70	.53	.20	.81	.52	.33	00	.11	.81	.44	.16	.97

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 — 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 — 15.56	20/20	25/25	30/30	35/35
0.9300	52.11	50.81	49.44	48.16	46.97	0.9230	55.52	54.24	52.88	51.61	50.41
0.9299	.16	.86	.49	.21	47.02	29	.57	.29	.93	.66	.46
98	.21	.91	.54	.26	.07	28	.62	.33	.98	.71	.51
97	.26	.96	.59	.31	.12	27	.67	.38	53.03	.75	.56
96	.31	51.01	.64	.36	.17	26	.71	.43	.08	.80	.60
95	.36	.06	.69	.41	.22	25	.76	.48	.12	.85	.65
94	.41	.11	.74	.46	.27	24	.81	.53	.17	.90	.70
93	.46	.16	.79	.51	.32	23	.86	.57	.22	.95	.75
92	.51	.21	.84	.56	.37	22	.90	.62	.27	52.00	.80
91	.56	.26	.89	.61	.42	21	.95	.67	.31	.04	.85
90	.61	.31	.94	.66	.47	20	56.00	.72	.36	.09	.89
89	.66	.36	.99	.71	.52	19	.05	.77	.41	.14	.94
88	.71	.41	50.04	.76	.57	18	.09	.81	.46	.19	.99
87	.76	.46	.09	.81	.62	17	.14	.86	.50	.23	51.04
86	.81	.50	.14	.86	.67	16	.19	.91	.55	.28	.09
85	.86	.55	.19	.91	.72	15	.24	.96	.60	.33	.13
84	.91	.60	.24	.96	.77	14	.28	55.00	.65	.38	.18
83	.96	.65	.29	49.01	.82	13	.33	.05	.70	.43	.23
82	53.00	.70	.34	.06	.87	12	.38	.10	.74	.47	.27
81	.05	.75	.39	.11	.92	11	.43	.15	.79	.52	.32
80	.10	.80	.44	.16	.97	10	.47	.19	.84	.57	.37
79	.15	.85	.49	.21	48.02	09	.52	.24	.89	.62	.42
78	.20	.90	.54	.26	.07	08	.57	.29	.93	.67	.46
77	.25	.95	.59	.31	.12	07	.62	.34	.98	.71	.51
76	.30	52.00	.64	.36	.17	06	.66	.38	54.03	.76	.56
75	.35	.05	.68	.41	.22	05	.71	.43	.08	.81	.61
74	.40	.10	.73	.46	.27	04	.76	.48	.12	.86	.65
73	.45	.15	.78	.51	.32	03	.81	.53	.17	.90	.70
72	.50	.20	.83	.56	.37	02	.85	.57	.22	.95	.75
71	.54	.25	.88	.61	.42	01	.90	.62	.26	53.00	.80
70	.59	.29	.93	.66	.47	00	.95	.67	.31	.05	.84
69	.64	.34	.98	.71	.52	0.9199	57.00	.71	.36	.09	.89
68	.69	.39	51.03	.76	.57	98	.04	.76	.41	.14	.94
67	.74	.44	.08	.81	.62	97	.09	.81	.45	.19	.99
66	.79	.49	.13	.86	.67	96	.13	.86	.50	.23	52.03
65	.84	.54	.18	.91	.71	95	.18	.90	.55	.28	.08
64	.89	.59	.23	.96	.76	94	.23	.95	.59	.33	.13
63	.94	.64	.27	50.00	.81	93	.27	56.00	.64	.37	.17
62	.99	.69	.32	.05	.86	92	.32	.04	.69	.42	.22
61	54.03	.74	.37	.10	.91	91	.37	.09	.74	.47	.27
60	.08	.79	.42	.15	.96	90	.41	.14	.78	.51	.32
59	.13	.84	.47	.20	49.01	89	.46	.18	.83	.56	.36
58	.18	.89	.52	.25	.06	88	.51	.23	.88	.61	.41
57	.23	.93	.57	.30	.11	87	.55	.28	.92	.65	.46
56	.28	.98	.62	.35	.15	86	.60	.32	.97	.70	.50
55	.32	53.03	.67	.40	.20	85	.65	.37	55.02	.75	.55
54	.37	.08	.72	.44	.25	84	.69	.42	.07	.79	.60
53	.42	.13	.76	.49	.30	83	.74	.46	.11	.84	.65
52	.47	.18	.81	.54	.35	82	.79	.51	.16	.89	.69
51	.52	.22	.86	.59	.40	81	.83	.56	.21	.93	.74
50	.57	.27	.91	.64	.44	80	.88	.60	.25	.98	.79
49	.61	.32	.96	.69	.49	79	.93	.65	.30	54.03	.83
48	.66	.37	52.01	.74	.54	78	.97	.70	.35	.07	.88
47	.71	.42	.06	.79	.59	77	58.02	.74	.39	.12	.93
46	.76	.47	.11	.83	.64	76	.06	.79	.44	.17	.98
45	.81	.52	.16	.88	.69	75	.11	.84	.49	.21	53.02
44	.86	.56	.20	.93	.73	74	.16	.88	.53	.26	.07
43	.90	.61	.25	.98	.78	73	.20	.93	.58	.31	.12
42	.95	.66	.30	51.03	.83	72	.25	.97	.63	.36	.16
41	55.00	.71	.35	.08	.88	71	.29	57.02	.67	.40	.21
40	.05	.76	.40	.13	.93	70	.34	.07	.72	.45	.26
39	.10	.81	.45	.17	.98	69	.38	.11	.77	.50	.30
38	.14	.85	.50	.22	50.02	68	.43	.16	.81	.54	.35
37	.19	.90	.54	.27	.07	67	.47	.21	.86	.59	.40
36	.24	.95	.59	.32	.12	66	.52	.25	.91	.64	.44
35	.29	54.00	.64	.37	.17	65	.57	.30	.95	.68	.49
34	.33	.05	.69	.42	.22	64	.61	.35	56.00	.73	.53
33	.38	.09	.74	.46	.27	63	.66	.39	.05	.78	.58
32	.43	.14	.79	.51	.31	62	.70	.44	.09	.82	.63
31	.48	.19	.83	.56	.36	61	.75	.48	.14	.87	.67
30	.52	.24	.88	.61	.41	60	.79	.53	.18	.92	.72

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 19

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9160	58.79	57.53	56.18	54.92	53.72	0.9090	61.92	60.68	59.36	58.11	56.93
59	.84	.58	.23	.96	.77	89	.96	.72	.40	.15	.97
58	.89	.62	.28	55.01	.81	88	62.01	.77	.45	.20	57.02
57	.93	.67	.32	.06	.86	87	.05	.81	.49	.24	.06
56	.98	.71	.37	.10	.91	86	.10	.86	.54	.29	.11
55	59.02	.76	.41	.15	.95	85	.14	.90	.58	.33	.15
54	.07	.81	.46	.19	54.00	84	.18	.94	.63	.38	.19
53	.11	.85	.51	.24	.05	83	.23	.99	.67	.42	.24
52	.16	.90	.55	.29	.09	82	.27	61.03	.71	.46	.28
51	.20	.94	.60	.33	.14	81	.31	.08	.76	.51	.33
50	.25	.99	.65	.38	.18	80	.36	.12	.80	.55	.37
49	.29	58.03	.69	.42	.23	79	.40	.17	.85	.60	.42
48	.34	.08	.74	.47	.28	78	.45	.21	.89	.64	.46
47	.38	.13	.78	.52	.32	77	.49	.25	.94	.69	.50
46	.43	.17	.83	.56	.37	76	.53	.30	.98	.73	.55
45	.47	.22	.88	.61	.41	75	.58	.34	60.03	.77	.59
44	.52	.26	.92	.65	.46	74	.62	.39	.07	.82	.64
43	.56	.31	.97	.70	.51	73	.66	.43	.11	.86	.68
42	.61	.35	57.01	.75	.55	72	.71	.47	.16	.91	.73
41	.65	.40	.06	.79	.60	71	.75	.52	.20	.95	.77
40	.70	.44	.10	.84	.65	70	.79	.56	.25	59.00	.81
39	.74	.49	.15	.88	.69	69	.84	.60	.29	.04	.86
38	.79	.53	.20	.93	.74	68	.88	.65	.33	.08	.90
37	.83	.58	.24	.98	.78	67	.93	.69	.38	.13	.95
36	.88	.62	.29	56.02	.83	66	.97	.74	.42	.17	.99
35	.92	.67	.33	.07	.88	65	63.01	.78	.46	.21	58.04
34	.97	.71	.38	.11	.92	64	.06	.82	.51	.26	.08
33	60.01	.76	.42	.16	.97	63	.10	.87	.55	.30	.12
32	.06	.80	.47	.21	55.01	62	.14	.91	.60	.35	.17
31	.10	.85	.51	.25	.06	61	.19	.96	.64	.39	.21
30	.15	.89	.56	.30	.11	60	.23	62.00	.68	.43	.26
29	.19	.94	.60	.34	.15	59	.27	.04	.73	.48	.30
28	.24	.98	.65	.39	.20	58	.32	.09	.77	.52	.34
27	.28	59.03	.70	.44	.24	57	.36	.13	.82	.57	.39
26	.33	.07	.74	.48	.29	56	.40	.17	.86	.61	.43
25	.37	.12	.79	.53	.33	55	.45	.22	.90	.65	.48
24	.42	.16	.83	.57	.38	54	.49	.26	.95	.70	.52
23	.46	.21	.88	.62	.42	53	.53	.30	.99	.74	.56
22	.50	.25	.92	.67	.47	52	.58	.35	61.03	.79	.61
21	.55	.30	.97	.71	.52	51	.62	.39	.08	.83	.65
20	.59	.34	58.01	.76	.56	50	.66	.43	.12	.87	.70
19	.64	.39	.06	.80	.61	49	.71	.48	.16	.92	.74
18	.68	.43	.10	.85	.65	48	.75	.52	.21	.96	.78
17	.73	.48	.15	.89	.70	47	.79	.56	.25	60.00	.83
16	.77	.52	.19	.94	.74	46	.84	.60	.29	.05	.87
15	.82	.57	.24	.99	.79	45	.88	.65	.34	.09	.92
14	.86	.61	.28	57.03	.84	44	.92	.69	.38	.14	.96
13	.91	.66	.33	.08	.88	43	.97	.73	.42	.18	59.00
12	.95	.70	.37	.12	.93	42	64.01	.78	.47	.22	.05
11	61.00	.75	.42	.17	.97	41	.05	.82	.51	.27	.09
10	.04	.79	.46	.21	56.02	40	.09	.86	.55	.31	.13
09	.08	.84	.51	.26	.06	39	.14	.91	.60	.35	.18
08	.13	.88	.55	.30	.11	38	.18	.95	.64	.40	.22
07	.17	.92	.60	.35	.15	37	.22	.99	.68	.44	.27
06	.22	.97	.64	.39	.20	36	.27	63.04	.73	.48	.31
05	.26	60.01	.69	.44	.25	35	.31	.08	.77	.53	.35
04	.30	.06	.73	.48	.29	34	.35	.12	.81	.57	.40
03	.35	.10	.78	.53	.34	33	.40	.17	.86	.62	.44
02	.39	.15	.82	.57	.38	32	.44	.21	.90	.66	.48
01	.44	.19	.87	.62	.43	31	.48	.25	.94	.70	.53
00	.48	.24	.91	.66	.47	30	.53	.30	.99	.75	.57
0.9099	.52	.28	.96	.71	.52	29	.57	.34	62.03	.79	.61
98	.57	.33	59.00	.75	.56	28	.61	.38	.07	.83	.66
97	.61	.37	.04	.80	.61	27	.66	.43	.12	.88	.70
96	.66	.41	.09	.84	.65	26	.70	.47	.16	.92	.74
95	.70	.46	.13	.89	.70	25	.74	.51	.20	.97	.79
94	.74	.50	.18	.93	.75	24	.78	.56	.24	61.01	.83
93	.79	.55	.22	.98	.79	23	.83	.60	.29	.05	.87
92	.83	.59	.27	58.02	.84	22	.87	.64	.23	.09	.92
91	.88	.64	.31	.07	.88	21	.91	.69	.37	.14	.96
90	.92	.68	.36	.11	.93	20	.96	.73	.42	.18	60.00

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9020	64.96	63.73	62.42	61.18	60.00	0.8950	67.90	66.69	65.39	64.16	62.99
19	65.00	.77	.46	.22	.05	49	.94	.73	.44	.21	63.03
18	.04	.82	.50	.27	.09	48	.98	.77	.48	.25	.08
17	.09	.86	.55	.31	.13	47	68.02	.81	.52	.29	.12
16	.13	.90	.59	.35	.18	46	.07	.85	.56	.33	.16
15	.17	.94	.63	.40	.22	45	.11	.90	.60	.37	.20
14	.21	.99	.67	.44	.26	44	.15	.94	.64	.42	.24
13	.26	64.03	.72	.48	.30	43	.19	.98	.69	.46	.28
12	.30	.07	.76	.53	.35	42	.23	67.02	.73	.50	.33
11	.34	.11	.80	.57	.39	41	.27	.06	.77	.54	.37
10	.38	.16	.85	.61	.43	40	.31	.10	.81	.58	.41
09	.43	.20	.89	.66	.48	39	.35	.15	.85	.63	.45
08	.47	.24	.93	.70	.52	38	.39	.19	.90	.67	.49
07	.51	.28	.97	.74	.56	37	.43	.23	.94	.71	.54
06	.55	.33	63.02	.78	.61	36	.48	.27	.98	.75	.58
05	.60	.37	.06	.83	.65	35	.52	.31	66.02	.79	.62
04	.64	.41	.10	.87	.69	34	.56	.35	.06	.84	.66
03	.68	.45	.15	.91	.73	33	.60	.39	.10	.88	.70
02	.72	.50	.19	.96	.78	32	.64	.43	.15	.92	.74
01	.77	.54	.23	62.00	.82	31	.68	.47	.19	.96	.79
00	.81	.58	.27	.04	.86	30	.72	.52	.23	65.00	.83
0.8999	.85	.62	.32	.09	.91	29	.76	.56	.27	.05	.87
98	.89	.67	.36	.13	.95	28	.80	.60	.31	.09	.91
97	.94	.71	.40	.17	.99	27	.84	.64	.35	.13	.95
96	.98	.75	.44	.21	61.03	26	.89	.68	.39	.17	64.00
95	66.02	.79	.49	.26	.08	25	.93	.72	.44	.21	.04
94	.06	.84	.53	.30	.12	24	.97	.76	.48	.25	.08
93	.10	.88	.57	.34	.16	23	69.01	.80	.52	.29	.12
92	.15	.92	.62	.38	.21	22	.05	.84	.56	.34	.16
91	.19	.96	.66	.43	.25	21	.09	.89	.60	.38	.21
90	.23	65.01	.70	.47	.29	20	.13	.93	.64	.42	.25
89	.27	.05	.74	.51	.33	19	.17	.97	.68	.46	.29
88	.31	.09	.79	.55	.38	18	.21	68.01	.73	.50	.33
87	.36	.13	.83	.60	.42	17	.25	.05	.77	.54	.37
86	.40	.18	.87	.64	.46	16	.29	.09	.81	.59	.41
85	.44	.22	.91	.68	.50	15	.33	.13	.85	.63	.46
84	.48	.26	.96	.72	.55	14	.37	.17	.89	.67	.50
83	.52	.30	64.00	.77	.59	13	.41	.21	.93	.71	.54
82	.56	.35	.04	.81	.63	12	.46	.26	.98	.75	.58
81	.61	.39	.08	.85	.68	11	.50	.30	67.02	.79	.62
80	.65	.43	.13	.89	.72	10	.54	.34	.06	.83	.67
79	.69	.47	.17	.94	.76	09	.58	.38	.10	.88	.71
78	.73	.51	.21	.98	.80	08	.62	.42	.14	.92	.75
77	.77	.56	.25	63.02	.85	07	.66	.46	.18	.96	.79
76	.82	.60	.30	.06	.89	06	.70	.50	.22	66.00	.83
75	.86	.64	.34	.11	.93	05	.74	.54	.26	.04	.87
74	.90	.68	.38	.15	.97	04	.78	.58	.30	.08	.92
73	.94	.72	.42	.19	62.02	03	.82	.62	.34	.12	.96
72	.98	.77	.47	.23	.06	02	.86	.67	.39	.17	65.00
71	67.03	.81	.51	.28	.10	01	.90	.71	.43	.21	.04
70	.07	.85	.55	.32	.14	00	.94	.75	.47	.25	.08
69	.11	.89	.59	.36	.19	0.8899	.98	.79	.51	.29	.12
68	.15	.94	.64	.40	.23	98	70.02	.83	.55	.33	.17
67	.19	.98	.68	.44	.27	97	.06	.87	.59	.37	.21
66	.23	66.02	.72	.49	.31	96	.10	.91	.63	.41	.25
65	.28	.06	.76	.53	.36	95	.14	.95	.67	.45	.29
64	.32	.10	.81	.57	.40	94	.18	.99	.71	.50	.33
63	.36	.15	.85	.61	.44	93	.22	69.03	.75	.54	.37
62	.40	.19	.89	.66	.48	92	.27	.07	.80	.58	.41
61	.44	.23	.93	.70	.53	91	.31	.11	.84	.62	.45
60	.48	.27	.97	.74	.57	90	.35	.15	.88	.66	.50
59	.53	.31	65.02	.78	.61	89	.39	.19	.92	.70	.54
58	.57	.36	.06	.83	.65	88	.43	.23	.96	.74	.58
57	.61	.40	.10	.87	.69	87	.47	.27	68.00	.79	.62
56	.65	.44	.14	.91	.74	86	.51	.32	.04	.83	.66
55	.69	.48	.18	.95	.78	85	.55	.36	.08	.87	.70
54	.73	.52	.23	64.00	.82	84	.59	.40	.12	.91	.74
53	.78	.56	.27	.04	.86	83	.63	.44	.16	.95	.79
52	.82	.60	.31	.08	.91	82	.67	.48	.20	.99	.83
51	.86	.65	.35	.12	.95	81	.71	.52	.24	67.03	.87
50	.90	.69	.39	.16	.99	80	.75	.56	.28	.07	.91

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 19

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8880	70.75	69.56	68.28	67.07	65.91	0.8810	73.50	72.34	71.09	69.89	68.74
79	.79	.60	.33	.11	.95	09	.54	.38	.13	.93	.78
78	.83	.64	.37	.15	.99	08	.58	.42	.16	.97	.82
77	.87	.68	.41	.20	66.03	07	.62	.46	.20	70.01	.86
76	.91	.72	.45	.24	.07	06	.66	.50	.24	.05	.90
75	.95	.76	.49	.28	.11	05	.70	.53	.28	.09	.94
74	.99	.80	.53	.32	.16	04	.74	.57	.32	.13	.98
73	71.03	.84	.57	.36	.20	03	.78	.61	.36	.17	69.02
72	.07	.88	.61	.40	.24	02	.81	.65	.40	.21	.06
71	.11	.92	.65	.44	.28	01	.85	.69	.44	.25	.10
70	.15	.96	.69	.48	.32	00	.89	.73	.48	.29	.14
69	.19	70.00	.73	.52	.36	0.8799	.93	.77	.52	.33	.18
68	.23	.04	.77	.56	.40	98	.97	.81	.56	.37	.22
67	.27	.08	.81	.60	.44	97	74.01	.85	.60	.41	.26
66	.31	.12	.85	.64	.48	96	.05	.88	.64	.44	.30
65	.35	.16	.89	.68	.52	95	.08	.92	.67	.48	.34
64	.38	.20	.93	.72	.56	94	.12	.96	.71	.52	.38
63	.42	.24	.98	.76	.60	93	.16	73.00	.75	.56	.42
62	.46	.28	69.02	.80	.64	92	.20	.04	.79	.60	.45
61	.50	.32	.06	.85	.69	91	.24	.08	.83	.64	.49
60	.54	.36	.10	.89	.73	90	.28	.12	.87	.68	.53
59	.58	.40	.14	.93	.77	89	.32	.16	.91	.72	.57
58	.62	.44	.18	.97	.81	88	.36	.19	.95	.76	.61
57	.66	.48	.22	68.01	.85	87	.39	.23	.99	.80	.65
56	.70	.52	.26	.05	.89	86	.43	.27	72.03	.84	.69
55	.74	.56	.30	.09	.93	85	.47	.31	.07	.88	.73
54	.78	.60	.34	.13	.97	84	.51	.35	.11	.92	.77
53	.82	.64	.38	.17	67.01	83	.55	.39	.14	.96	.81
52	.86	.68	.42	.21	.05	82	.59	.43	.18	71.00	.85
51	.90	.72	.46	.25	.09	81	.63	.47	.22	.04	.89
50	.94	.76	.50	.29	.13	80	.66	.50	.26	.07	.93
49	.98	.80	.54	.33	.17	79	.70	.54	.30	.11	.97
48	72.02	.84	.58	.37	.21	78	.74	.58	.34	.15	70.01
47	.10	.88	.62	.41	.25	77	.78	.62	.38	.19	.05
46	.14	.92	.66	.45	.29	76	.82	.66	.42	.23	.09
45	.18	.96	.70	.49	.33	75	.86	.70	.46	.27	.13
44	.22	71.00	.74	.53	.38	74	.90	.74	.49	.31	.16
43	.26	.04	.78	.57	.42	73	.93	.78	.53	.35	.20
42	.30	.08	.82	.61	.46	72	.97	.81	.57	.39	.24
41	.34	.12	.86	.65	.50	71	75.01	.85	.61	.42	.28
40	.38	.16	.90	.69	.54	70	.05	.89	.65	.46	.32
39	.42	.20	.94	.73	.58	69	.09	.93	.69	.50	.36
38	.46	.24	.98	.77	.62	68	.13	.97	.73	.54	.40
37	.50	.28	70.02	.81	.66	67	.16	74.01	.77	.58	.44
36	.54	.32	.06	.85	.70	66	.20	.05	.81	.62	.48
35	.58	.36	.10	.89	.74	65	.24	.08	.84	.66	.52
34	.62	.40	.14	.93	.78	64	.28	.12	.88	.70	.56
33	.66	.44	.18	.97	.82	63	.32	.16	.92	.74	.60
32	.70	.48	.22	69.01	.86	62	.35	.20	.96	.77	.64
31	.74	.52	.26	.05	.90	61	.39	.24	73.00	.81	.67
30	.78	.56	.30	.09	.94	60	.43	.28	.04	.85	.71
29	.82	.60	.34	.13	.98	59	.47	.32	.08	.89	.75
28	.86	.64	.38	.17	68.02	58	.51	.35	.12	.93	.79
27	.90	.68	.42	.21	.06	57	.54	.39	.15	.97	.83
26	.94	.72	.46	.25	.10	56	.58	.43	.19	72.01	.87
25	.98	.76	.50	.29	.14	55	.62	.47	.23	.05	.91
24	.02	.80	.54	.33	.18	54	.66	.51	.27	.08	.95
23	73.00	.84	.58	.37	.22	53	.70	.55	.31	.12	.99
22	.04	.88	.62	.41	.26	52	.74	.58	.35	.16	71.03
21	.08	.92	.66	.45	.30	51	.78	.62	.38	.20	.07
20	.12	.96	.70	.49	.34	50	.82	.66	.42	.24	.10
19	.16	.00	.74	.53	.38	49	.86	.70	.46	.28	.14
18	.20	72.03	.78	.57	.42	48	.89	.74	.50	.32	.18
17	.24	.04	.82	.61	.46	47	.92	.77	.54	.36	.22
16	.28	.08	.86	.65	.50	46	.96	.81	.58	.39	.26
15	.32	.12	.90	.69	.54	45	76.00	.85	.62	.43	.30
14	.36	.16	.94	.73	.58	44	.04	.89	.65	.47	.34
13	.40	.20	.98	.77	.62	43	.07	.93	.69	.51	.38
12	.44	.24	71.01	.81	.66	42	.11	.97	.73	.55	.41
11	.48	.28	.05	.85	.70	41	.15	75.00	.77	.59	.45
10	.52	.32	.09	.89	.74	40	.19	.04	.81	.63	.49

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8740	76.19	75.04	73.81	72.63	71.49	0.8670	78.78	77.66	76.45	75.29	74.17
39	.22	.08	.85	.66	.53	69	.82	.70	.49	.33	.21
38	.26	.12	.88	.70	.57	68	.85	.73	.53	.37	.24
37	.30	.16	.92	.74	.61	67	.89	.77	.56	.40	.28
36	.34	.19	.96	.78	.65	66	.93	.81	.60	.44	.32
35	.37	.23	74.00	.82	.69	65	.96	.84	.64	.48	.36
34	.41	.27	.04	.86	.72	64	79.00	.88	.68	.51	.39
33	.45	.31	.08	.90	.76	63	.04	.92	.71	.55	.43
32	.49	.35	.11	.93	.80	62	.07	.96	.75	.59	.47
31	.52	.38	.15	.97	.84	61	.11	.99	.79	.63	.51
30	.56	.42	.19	73.01	.88	60	.14	78.03	.82	.66	.55
29	.60	.46	.23	.05	.92	59	.18	.07	.86	.70	.58
28	.64	.50	.27	.09	.96	58	.22	.10	.90	.74	.62
27	.67	.54	.31	.13	72.00	57	.25	.14	.94	.78	.66
26	.71	.57	.34	.16	.03	56	.29	.17	.97	.81	.70
25	.75	.61	.38	.20	.07	55	.32	.21	77.01	.85	.73
24	.79	.65	.42	.24	.11	54	.36	.25	.05	.89	.77
23	.82	.69	.46	.28	.15	53	.40	.28	.08	.93	.81
22	.86	.73	.50	.32	.19	52	.43	.32	.12	.96	.85
21	.90	.76	.53	.35	.23	51	.47	.36	.16	76.00	.88
20	.94	.80	.57	.39	.27	50	.51	.39	.19	.04	.92
19	.97	.84	.61	.43	.30	49	.54	.43	.23	.07	.96
18	77.01	.88	.65	.47	.34	48	.58	.47	.27	.11	75.00
17	.05	.91	.69	.51	.38	47	.61	.50	.30	.15	.03
16	.09	.95	.73	.55	.42	46	.65	.54	.34	.19	.07
15	.12	.99	.76	.58	.46	45	.69	.57	.38	.22	.11
14	.16	76.03	.80	.62	.50	44	.72	.61	.41	.26	.15
13	.20	.06	.84	.66	.53	43	.76	.65	.45	.30	.18
12	.23	.10	.88	.70	.57	42	.79	.68	.49	.33	.22
11	.27	.14	.92	.74	.61	41	.83	.72	.52	.37	.26
10	.31	.18	.95	.77	.65	40	.87	.76	.56	.41	.29
09	.34	.22	.99	.81	.69	39	.90	.79	.60	.44	.33
08	.38	.25	75.03	.85	.73	38	.94	.83	.63	.48	.37
07	.42	.29	.07	.89	.77	37	.97	.86	.67	.52	.41
06	.46	.33	.10	.93	.80	36	80.01	.90	.71	.56	.44
05	.49	.37	.14	.97	.84	35	.05	.94	.74	.59	.48
04	.53	.40	.18	74.00	.88	34	.08	.97	.78	.63	.52
03	.57	.44	.22	.04	.92	33	.12	79.01	.82	.67	.56
02	.60	.48	.25	.08	.96	32	.15	.05	.85	.70	.59
01	.64	.52	.29	.12	73.00	31	.19	.08	.89	.74	.63
00	.68	.55	.33	.16	.03	30	.22	.12	.93	.78	.67
0.8699	.71	.59	.37	.19	.07	29	.26	.16	.96	.81	.71
98	.75	.63	.40	.23	.11	28	.30	.19	78.00	.85	.74
97	.79	.66	.44	.27	.15	27	.33	.23	.04	.89	.78
96	.83	.70	.48	.31	.19	26	.37	.26	.07	.93	.82
95	.86	.74	.52	.35	.22	25	.40	.30	.11	.96	.85
94	.90	.78	.55	.38	.26	24	.44	.34	.14	77.00	.89
93	.94	.81	.59	.42	.30	23	.47	.37	.18	.04	.93
92	.97	.85	.63	.46	.34	22	.51	.41	.22	.07	.97
91	78.01	.89	.67	.50	.38	21	.55	.45	.25	.11	76.00
90	.05	.92	.70	.54	.41	20	.58	.48	.29	.15	.04
89	.08	.96	.74	.57	.45	19	.62	.52	.33	.18	.08
88	.12	77.00	.78	.61	.49	18	.65	.55	.36	.22	.11
87	.16	.03	.82	.65	.53	17	.69	.59	.40	.26	.15
86	.19	.07	.85	.69	.56	16	.72	.63	.43	.29	.19
85	.23	.11	.89	.73	.60	15	.76	.66	.47	.33	.23
84	.27	.14	.93	.76	.64	14	.80	.70	.51	.36	.26
83	.30	.18	.97	.80	.68	13	.83	.73	.54	.40	.30
82	.34	.22	76.00	.84	.72	12	.87	.77	.58	.44	.34
81	.38	.26	.04	.88	.75	11	.90	.80	.62	.47	.37
80	.41	.29	.08	.92	.79	10	.94	.84	.65	.51	.41
79	.45	.33	.12	.95	.83	09	.97	.88	.69	.55	.45
78	.49	.37	.15	.99	.87	08	81.01	.91	.72	.58	.48
77	.52	.40	.19	75.03	.91	07	.05	.95	.76	.62	.52
76	.56	.44	.23	.07	.94	06	.08	.98	.80	.66	.56
75	.60	.48	.26	.10	.98	05	.12	80.02	.83	.69	.59
74	.63	.51	.30	.14	74.02	04	.15	.05	.87	.73	.63
73	.67	.55	.34	.18	.06	03	.19	.09	.91	.77	.67
72	.71	.59	.38	.22	.09	02	.22	.13	.94	.80	.70
71	.74	.62	.41	.25	.13	01	.26	.16	.98	.84	.74
70	.78	.66	.45	.29	.17	00	.29	.20	79.01	.88	.78



Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 19

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8600	81.29	80.20	79.01	77.88	76.78	0.8530	83.73	82.66	81.50	80.38	79.30
0.8599	.33	.23	.05	.91	.82		.77	.69	.54	.42	.34
98	.36	.27	.09	.95	.85	29	.80	.73	.57	.45	.38
97	.40	.30	.12	.99	.89	28	.84	.76	.61	.49	.41
96	.43	.34	.16	78.02	.93	27	.87	.80	.64	.52	.45
95	.47	.38	.19	.06	.96	26	.90	.83	.68	.56	.48
94	.51	.41	.23	.09	77.00	25	.94	.87	.71	.59	.52
93	.54	.45	.27	.13	.04	24	.97	.90	.75	.63	.55
92	.58	.48	.30	.17	.07	23	84.01	.94	.78	.66	.59
91	.61	.52	.34	.20	.11	22	.04	.97	.82	.70	.62
						21					
90	.65	.55	.37	.24	.14	20	.07	83.01	.85	.73	.66
89	.68	.59	.41	.27	.18	19	.11	.04	.89	.77	.70
88	.72	.62	.44	.31	.22	18	.14	.07	.92	.81	.73
87	.75	.66	.48	.35	.25	17	.18	.11	.96	.84	.77
86	.79	.69	.52	.38	.29	16	.21	.14	.99	.88	.80
85	.82	.73	.55	.42	.33	15	.24	.18	82.03	.91	.84
84	.86	.77	.59	.45	.36	14	.28	.21	.06	.95	.87
83	.89	.80	.62	.49	.40	13	.31	.25	.10	.98	.91
82	.93	.84	.66	.53	.43	12	.34	.28	.13	81.02	.94
81	.96	.87	.70	.56	.47	11	.38	.32	.16	.05	.98
80	82.00	.91	.73	.60	.51	10	.41	.35	.20	.09	80.01
79	.03	.94	.77	.64	.54	09	.45	.39	.23	.12	.05
78	.07	.98	.80	.67	.58	08	.48	.42	.27	.16	.08
77	.10	81.01	.84	.71	.62	07	.51	.45	.30	.19	.12
76	.14	.05	.87	.74	.65	06	.55	.49	.34	.23	.15
75	.17	.08	.91	.78	.69	05	.58	.52	.37	.26	.19
74	.21	.12	.95	.82	.72	04	.61	.56	.41	.30	.23
73	.24	.16	.98	.85	.76	03	.65	.59	.44	.33	.26
72	.28	.19	80.02	.89	.80	02	.68	.62	.47	.37	.30
71	.31	.23	.05	.92	.83	01	.72	.66	.51	.40	.33
70	.35	.26	.09	.96	.87	00	.75	.69	.54	.44	.37
69	.38	.30	.12	79.00	.91	0.8499	.78	.73	.58	.47	.40
68	.42	.33	.16	.03	.94	98	.82	.76	.61	.51	.44
67	.45	.37	.20	.07	.98	97	.85	.79	.65	.54	.47
66	.49	.40	.23	.10	78.01	96	.89	.83	.68	.57	.51
65	.52	.44	.27	.14	.05	95	.92	.86	.71	.61	.54
64	.56	.47	.30	.17	.09	94	.95	.90	.75	.64	.58
63	.59	.51	.34	.21	.12	93	.99	.93	.78	.68	.61
62	.63	.54	.37	.25	.16	92	85.02	.97	.82	.71	.65
61	.66	.58	.41	.28	.19	91	.05	84.00	.85	.75	.68
60	.70	.61	.44	.32	.23	90	.09	.03	.89	.78	.72
59	.73	.65	.48	.35	.27	89	.12	.07	.92	.82	.75
58	.77	.68	.51	.39	.30	88	.15	.10	.96	.85	.79
57	.80	.72	.55	.42	.34	87	.18	.14	.99	.89	.82
56	.84	.75	.59	.46	.37	86	.22	.17	83.02	.92	.86
55	.87	.79	.62	.49	.41	85	.25	.20	.06	.96	.89
54	.91	.82	.66	.53	.45	84	.28	.24	.09	.99	.93
53	.94	.86	.69	.57	.48	83	.32	.27	.13	82.03	.96
52	.98	.89	.73	.60	.52	82	.35	.31	.16	.06	81.00
51	83.01	.93	.76	.64	.55	81	.38	.34	.20	.10	.03
50	.96	.80	.67	.59	.50	80	.42	.37	.23	.13	.07
49	.08	82.00	.83	.71	.63	79	.45	.41	.26	.17	.10
48	.11	.03	.87	.74	.66	78	.48	.44	.30	.20	.14
47	.15	.07	.90	.78	.70	77	.51	.47	.33	.24	.17
46	.18	.10	.94	.81	.73	76	.55	.51	.37	.27	.21
45	.22	.14	.98	.85	.77	75	.58	.54	.40	.30	.24
44	.25	.17	81.01	.89	.81	74	.61	.57	.43	.34	.28
43	.29	.21	.05	.92	.84	73	.65	.61	.47	.37	.31
42	.32	.24	.08	.96	.88	72	.68	.64	.50	.41	.35
41	.35	.28	.12	.99	.91	71	.71	.67	.54	.44	.38
40	.39	.31	.15	80.03	.95	70	.75	.71	.57	.48	.42
39	.42	.35	.19	.06	.99	69	.78	.74	.61	.51	.45
38	.46	.38	.22	.10	79.02	68	.81	.78	.64	.55	.49
37	.49	.42	.25	.13	.06	67	.84	.81	.67	.58	.52
36	.53	.45	.29	.17	.09	66	.88	.84	.71	.62	.56
35	.56	.49	.30	.20	.13	65	.91	.88	.74	.65	.59
34	.59	.52	.36	.24	.16	64	.94	.91	.78	.69	.63
33	.63	.55	.40	.28	.20	63	.98	.94	.81	.72	.66
32	.66	.59	.43	.31	.23	62	86.01	.98	.85	.75	.70
31	.70	.62	.47	.35	.27	61	.04	85.01	.88	.79	.73
30	.73	.66	.50	.38	.30	60	.08	.04	.91	.82	.77

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8460	86.08	85.04	83.91	82.82	81.77	0.8390	88.33	87.33	86.24	85.18	84.16
59	.11	.08	.95	.86	.80	89	.36	.36	.28	.22	.19
58	.14	.11	.98	.89	.84	88	.39	.39	.31	.25	.22
57	.17	.14	84.02	.93	.87	87	.43	.43	.34	.28	.26
56	.21	.18	.05	.96	.91	86	.46	.46	.37	.31	.29
55	.24	.21	.08	83.00	.94	85	.49	.49	.40	.35	.32
54	.27	.24	.12	.03	.98	84	.52	.52	.44	.38	.36
53	.30	.28	.15	.06	82.01	83	.55	.55	.47	.41	.39
52	.34	.31	.18	.10	.04	82	.58	.58	.50	.45	.42
51	.37	.34	.22	.13	.08	81	.61	.62	.53	.48	.46
50	.40	.38	.25	.17	.11	80	.65	.65	.57	.51	.49
49	.43	.41	.29	.20	.15	79	.68	.68	.60	.54	.52
48	.47	.44	.32	.23	.18	78	.71	.71	.63	.58	.55
47	.50	.48	.35	.27	.22	77	.74	.74	.66	.61	.59
46	.53	.51	.39	.30	.25	76	.77	.78	.70	.64	.62
45	.57	.54	.42	.34	.28	75	.80	.81	.73	.68	.65
44	.60	.57	.45	.37	.32	74	.83	.84	.76	.71	.69
43	.63	.61	.49	.40	.35	73	.87	.87	.79	.74	.72
42	.66	.64	.52	.44	.39	72	.90	.90	.83	.77	.75
41	.70	.67	.55	.47	.42	71	.93	.94	.86	.81	.79
40	.73	.71	.59	.51	.46	70	.96	.97	.89	.84	.82
39	.76	.74	.62	.54	.49	69	.99	88.00	.92	.87	.85
38	.79	.77	.65	.57	.52	68	89.02	.03	.95	.90	.89
37	.83	.80	.69	.61	.56	67	.05	.06	.99	.94	.92
36	.86	.84	.72	.64	.59	66	.08	.09	87.02	.97	.95
35	.89	.87	.76	.68	.63	65	.11	.13	.05	86.00	.99
34	.92	.90	.79	.71	.66	64	.14	.16	.08	.04	85.02
33	.96	.94	.82	.74	.70	63	.18	.19	.11	.07	.05
32	.99	.97	.86	.78	.73	62	.21	.22	.15	.10	.08
31	87.02	86.00	.89	.81	.76	61	.24	.25	.18	.13	.12
30	.05	.03	.92	.85	.80	60	.27	.29	.21	.16	.15
29	.09	.07	.96	.88	.83	59	.30	.32	.24	.20	.18
28	.12	.10	.99	.91	.87	58	.33	.35	.27	.23	.22
27	.15	.13	85.02	.95	.90	57	.36	.38	.31	.26	.25
26	.18	.16	.06	.98	.93	56	.39	.41	.34	.29	.28
25	.22	.20	.09	84.02	.97	55	.42	.44	.37	.33	.31
24	.25	.23	.12	.05	83.00	54	.45	.47	.40	.36	.35
23	.28	.26	.16	.08	.04	53	.48	.50	.43	.39	.38
22	.31	.30	.19	.12	.07	52	.51	.54	.46	.42	.41
21	.34	.33	.22	.15	.11	51	.54	.57	.50	.45	.44
20	.38	.36	.25	.18	.14	50	.58	.60	.53	.49	.48
19	.41	.39	.29	.22	.17	49	.61	.63	.56	.52	.51
18	.44	.43	.32	.25	.21	48	.64	.66	.59	.55	.54
17	.47	.46	.35	.28	.24	47	.67	.69	.62	.58	.58
16	.50	.49	.39	.32	.28	46	.70	.72	.66	.62	.61
15	.54	.52	.42	.35	.31	45	.73	.75	.69	.65	.64
14	.57	.56	.45	.38	.34	44	.76	.79	.72	.68	.67
13	.60	.59	.49	.42	.38	43	.79	.82	.75	.71	.71
12	.63	.62	.52	.45	.41	42	.82	.85	.78	.75	.74
11	.67	.65	.55	.48	.45	41	.85	.88	.82	.78	.77
10	.70	.68	.59	.52	.48	40	.88	.91	.85	.81	.80
09	.73	.72	.62	.55	.51	39	.91	.94	.88	.84	.84
08	.76	.75	.65	.59	.55	38	.94	.97	.91	.87	.87
07	.79	.78	.69	.62	.58	37	.98	89.00	.94	.91	.90
06	.83	.81	.72	.65	.62	36	90.01	.04	.97	.94	.93
05	.86	.85	.75	.69	.65	35	.04	.07	88.01	.97	.97
04	.89	.88	.78	.72	.68	34	.07	.10	.04	87.00	86.00
03	.92	.91	.82	.75	.72	33	.10	.13	.07	.04	.03
02	.95	.94	.85	.79	.75	32	.13	.16	.10	.07	.06
01	.99	.98	.88	.82	.79	31	.16	.19	.13	.10	.10
00	88.02	87.01	.92	.85	.82	30	.19	.22	.16	.13	.13
0.8399	.05	.04	.95	.89	.85	29	.22	.25	.19	.16	.16
98	.08	.07	.98	.92	.89	28	.25	.28	.23	.19	.19
97	.11	.10	86.02	.95	.92	27	.28	.31	.26	.23	.23
96	.14	.14	.05	.99	.96	26	.31	.35	.29	.26	.26
95	.18	.17	.08	85.02	.99	25	.34	.38	.32	.29	.29
94	.21	.20	.11	.05	84.02	24	.37	.41	.35	.32	.32
93	.24	.23	.15	.09	.06	23	.40	.44	.38	.35	.35
92	.27	.27	.18	.12	.09	22	.43	.47	.41	.39	.39
91	.30	.30	.21	.15	.12	21	.46	.50	.45	.42	.42
90	.33	.33	.24	.18	.16	20	.49	.53	.48	.45	.45

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

19

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8320	90.49	89.53	88.48	87.45	86.45	0.8250	92.53	91.62	90.61	89.64	88.67
19	.51	.56	.51	.48	.48	49	.55	.64	.64	.67	.70
18	.54	.59	.54	.51	.52	48	.58	.67	.67	.70	.73
17	.57	.62	.57	.54	.55	47	.61	.70	.70	.73	.76
16	.60	.65	.60	.58	.58	46	.64	.73	.73	.76	.79
15	.63	.68	.64	.61	.61	45	.66	.76	.76	.79	.83
14	.66	.71	.67	.64	.65	44	.69	.79	.79	.82	.86
13	.69	.74	.70	.67	.68	43	.72	.82	.82	.85	.89
12	.72	.77	.73	.70	.71	42	.75	.85	.85	.88	.92
11	.75	.80	.76	.74	.74	41	.78	.87	.88	.91	.95
10	.78	.83	.79	.77	.77	40	.80	.90	.91	.94	.98
09	.81	.86	.82	.80	.81	39	.83	.93	.94	.97	99.01
08	.84	.89	.85	.83	.84	38	.86	.96	.97	90.00	.04
07	.87	.93	.88	.86	.87	37	.89	.99	91.00	.03	.07
06	.90	.96	.92	.89	.90	36	.92	92.02	.03	.06	.10
05	.93	.99	.95	.93	.94	35	.94	.05	.06	.09	.13
04	.96	90.02	.98	.96	.97	34	.97	.08	.09	.12	.16
03	.99	.05	89.01	.99	87.00	33	93.00	.10	.12	.15	.20
02	91.02	.08	.04	88.02	.03	32	.03	.13	.15	.18	.23
01	.05	.11	.07	.05	.06	31	.05	.16	.18	.21	.26
00	.08	.14	.10	.08	.10	30	.08	.19	.21	.24	.29
08299	.11	.17	.13	.12	.13	29	.11	.22	.23	.27	.32
98	.14	.20	.16	.15	.16	28	.14	.25	.26	.30	.35
97	.17	.23	.19	.18	.19	27	.16	.28	.29	.33	.38
96	.20	.26	.22	.21	.22	26	.19	.31	.32	.36	.41
95	.23	.29	.25	.24	.25	25	.22	.33	.35	.39	.44
94	.26	.32	.28	.27	.29	24	.25	.36	.38	.42	.47
93	.28	.35	.31	.30	.32	23	.27	.39	.41	.45	.50
92	.31	.38	.35	.34	.35	22	.30	.42	.44	.48	.53
91	.34	.41	.38	.37	.38	21	.33	.45	.47	.51	.56
90	.37	.44	.41	.40	.41	20	.36	.48	.50	.54	.59
89	.40	.47	.44	.43	.44	19	.38	.50	.52	.57	.62
88	.43	.50	.47	.46	.48	18	.41	.53	.55	.60	.65
87	.46	.53	.50	.49	.51	17	.44	.56	.58	.63	.68
86	.49	.56	.53	.52	.54	16	.47	.59	.61	.66	.71
85	.52	.59	.56	.55	.57	15	.49	.62	.64	.69	.74
84	.55	.62	.59	.59	.60	14	.52	.65	.67	.72	.77
83	.58	.65	.62	.62	.63	13	.55	.67	.70	.75	.80
82	.60	.67	.65	.65	.67	12	.58	.70	.73	.78	.84
81	.63	.70	.68	.68	.70	11	.60	.73	.76	.81	.87
80	.66	.73	.71	.71	.73	10	.63	.76	.79	.84	.90
79	.69	.76	.74	.74	.76	09	.66	.79	.81	.87	.93
78	.72	.79	.77	.77	.79	08	.68	.81	.84	.90	.96
77	.75	.82	.80	.81	.83	07	.71	.84	.87	.93	.99
76	.78	.85	.83	.84	.86	06	.74	.87	.90	.96	90.02
75	.81	.88	.87	.87	.89	05	.76	.90	.93	.99	.05
74	.84	.91	.90	.90	.92	04	.79	.92	.96	91.01	.08
73	.87	.94	.93	.93	.95	03	.82	.95	.99	.04	.11
72	.90	.97	.96	.96	.98	02	.84	.98	92.02	.07	.14
71	.92	91.00	.99	.99	88.02	01	.87	93.01	.05	.10	.17
70	.95	.03	90.02	89.02	.05	00	.90	.04	.07	.13	.20
69	.98	.06	.05	.06	.08	0.8199	.92	.06	.10	.16	.23
68	92.01	.09	.08	.09	.11	98	.95	.09	.13	.19	.26
67	.04	.12	.11	.12	.14	97	.98	.12	.16	.22	.29
66	.07	.15	.14	.15	.17	96	94.01	.14	.19	.25	.32
65	.10	.18	.17	.18	.20	95	.03	.17	.22	.27	.35
64	.13	.21	.20	.21	.23	94	.06	.20	.24	.30	.38
63	.15	.24	.23	.24	.26	93	.08	.23	.27	.33	.40
62	.18	.27	.26	.27	.30	92	.11	.25	.30	.36	.43
61	.21	.29	.29	.30	.33	91	.14	.28	.33	.39	.46
60	.24	.32	.32	.33	.36	90	.16	.31	.36	.42	.49
59	.27	.35	.35	.36	.39	89	.19	.34	.39	.45	.52
58	.30	.38	.38	.39	.42	88	.22	.36	.41	.48	.55
57	.33	.41	.41	.42	.45	87	.24	.39	.44	.51	.58
56	.35	.44	.44	.45	.48	86	.27	.42	.47	.53	.61
55	.38	.47	.47	.48	.51	85	.30	.44	.50	.56	.64
54	.41	.50	.50	.51	.55	84	.32	.47	.53	.59	.67
53	.44	.53	.53	.54	.58	83	.35	.50	.56	.62	.70
52	.47	.56	.56	.57	.61	82	.38	.53	.58	.65	.73
51	.50	.59	.59	.61	.64	81	.40	.55	.61	.68	.76
50	.53	.62	.61	.64	.67	80	.43	.58	.64	.71	.79

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8180	94.43	93.58	92.64	91.71	90.79	0.8110	96.20	95.42	94.53	93.67	92.80
79	.46	.61	.67	.74	.82	09	.23	.44	.56	.69	.83
78	.48	.64	.70	.77	.85	08	.25	.47	.59	.72	.86
77	.51	.66	.72	.79	.88	07	.28	.49	.61	.75	.89
76	.53	.69	.75	.82	.91	06	.30	.52	.64	.77	.92
75	.56	.72	.78	.85	.94	05	.32	.54	.66	.80	.94
74	.59	.74	.81	.88	.97	04	.35	.57	.69	.83	.97
73	.61	.77	.84	.91	91.00	03	.37	.59	.72	.85	93.00
72	.64	.80	.86	.94	.03	02	.40	.62	.74	.88	.03
71	.66	.82	.89	.97	.06	01	.42	.64	.77	.91	.05
70	.69	.85	.92	92.00	.09	00	.45	.67	.79	.94	.08
69	.72	.88	.95	.03	.12	0.8099	.47	.69	.82	.96	.11
68	.74	.90	.97	.05	.14	98	.50	.72	.85	.99	.14
67	.77	.93	93.00	.08	.17	97	.52	.74	.87	94.02	.16
66	.79	.96	.03	.11	.20	96	.54	.77	.90	.04	.19
65	.82	.98	.06	.14	.23	95	.57	.79	.92	.07	.22
64	.84	94.01	.09	.17	.26	94	.59	.82	.95	.10	.25
63	.87	.04	.11	.20	.29	93	.61	.84	.98	.12	.27
62	.90	.06	.14	.22	.32	92	.64	.87	95.00	.15	.30
61	.92	.09	.17	.25	.35	91	.66	.89	.03	.17	.33
60	.95	.12	.20	.28	.38	90	.69	.92	.05	.20	.36
59	.97	.14	.22	.31	.40	89	.71	.94	.08	.23	.38
58	95.00	.17	.25	.34	.43	88	.73	.97	.10	.25	.41
57	.03	.20	.28	.36	.46	87	.76	.99	.13	.28	.44
56	.05	.22	.30	.39	.49	86	.78	96.02	.16	.31	.46
55	.08	.25	.33	.42	.52	85	.81	.04	.18	.33	.49
54	.10	.28	.36	.45	.55	84	.83	.07	.21	.36	.52
53	.13	.30	.39	.48	.58	83	.85	.09	.23	.39	.55
52	.15	.33	.41	.51	.61	82	.88	.11	.26	.41	.57
51	.18	.36	.44	.54	.64	81	.90	.14	.28	.44	.60
50	.20	.38	.47	.56	.66	80	.93	.16	.31	.47	.63
49	.23	.41	.50	.59	.69	79	.95	.19	.33	.49	.65
48	.25	.44	.52	.62	.72	78	.97	.21	.36	.52	.68
47	.28	.46	.55	.65	.75	77	97.00	.24	.39	.54	.71
46	.30	.49	.58	.68	.78	76	.02	.26	.41	.57	.73
45	.33	.51	.60	.70	.81	75	.04	.29	.44	.60	.76
44	.36	.54	.63	.73	.84	74	.07	.31	.46	.62	.79
43	.38	.57	.66	.76	.87	73	.09	.33	.49	.65	.81
42	.41	.59	.69	.79	.90	72	.11	.36	.51	.67	.84
41	.43	.62	.71	.82	.92	71	.14	.38	.54	.70	.87
40	.46	.64	.74	.84	.95	70	.16	.41	.56	.73	.90
39	.48	.67	.77	.87	.98	69	.18	.43	.59	.75	.92
38	.51	.70	.79	.90	92.01	68	.21	.46	.61	.78	.95
37	.53	.72	.82	.93	.04	67	.23	.48	.64	.80	.98
36	.56	.75	.85	.96	.07	66	.25	.50	.66	.83	94.00
35	.58	.77	.87	.98	.10	65	.28	.53	.69	.86	.03
34	.61	.80	.90	93.01	.13	64	.30	.55	.71	.88	.06
33	.63	.83	.93	.04	.15	63	.32	.58	.74	.91	.08
32	.66	.85	.95	.07	.18	62	.35	.60	.76	.93	.11
31	.68	.88	.98	.09	.21	61	.37	.63	.79	.96	.14
30	.71	.90	94.01	.12	.24	60	.39	.65	.81	.99	.16
29	.73	.93	.03	.15	.27	59	.42	.67	.84	95.01	.19
28	.76	.95	.06	.18	.30	58	.44	.70	.86	.04	.22
27	.78	.98	.09	.20	.33	57	.46	.72	.89	.06	.24
26	.81	95.01	.11	.23	.35	56	.49	.75	.91	.09	.27
25	.83	.03	.14	.26	.38	55	.51	.77	.94	.11	.30
24	.86	.06	.17	.29	.41	54	.53	.79	.96	.14	.32
23	.88	.08	.19	.31	.44	53	.55	.82	.99	.17	.35
22	.91	.11	.22	.34	.47	52	.58	.84	96.01	.19	.38
21	.93	.14	.24	.37	.50	51	.60	.86	.04	.22	.40
20	.96	.16	.27	.40	.53	50	.62	.89	.06	.24	.43
19	.98	.19	.30	.42	.55	49	.64	.91	.09	.27	.45
18	96.01	.21	.32	.45	.58	48	.67	.94	.11	.29	.48
17	.03	.24	.35	.48	.61	47	.69	.96	.14	.32	.51
16	.06	.26	.38	.51	.64	46	.71	.98	.16	.34	.53
15	.08	.29	.40	.53	.66	45	.73	97.01	.19	.37	.56
14	.10	.32	.43	.56	.69	44	.76	.03	.21	.40	.59
13	.13	.34	.46	.59	.72	43	.78	.05	.24	.42	.61
12	.15	.37	.48	.61	.75	42	.80	.08	.26	.45	.64
11	.18	.39	.51	.64	.78	41	.82	.10	.28	.47	.66
10	.20	.42	.53	.67	.80	40	.85	.12	.31	.50	.69

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

19

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
	15.56						15.56				
0.8040	97.85	97.12	96.31	95.50	94.69	0.7970	99.33	98.68	97.95	97.21	96.46
39	.87	.15	.33	.52	.72	69	.35	.70	.97	.23	.48
38	.89	.17	.36	.55	.74	68	.37	.72	.99	.25	.51
37	.91	.19	.38	.57	.77	67	.39	.75	98.02	.28	.53
36	.94	.22	.41	.60	.79	66	.42	.77	.04	.30	.56
35	.96	.24	.43	.62	.82	65	.44	.79	.06	.32	.58
34	.98	.26	.46	.65	.85	64	.46	.81	.08	.35	.60
33	98.00	.29	.48	.67	.87	63	.48	.83	.10	.37	.63
32	.03	.31	.50	.70	.90	62	.50	.85	.12	.39	.65
31	.05	.33	.53	.72	.92	61	.52	.87	.15	.42	.68
30	.07	.36	.55	.75	.95	60	.54	.89	.17	.44	.70
29	.09	.38	.58	.77	.98	59	.56	.91	.19	.46	.72
28	.11	.40	.60	.80	95.00	58	.58	.93	.21	.49	.75
27	.14	.43	.62	.82	.03	57	.60	.95	.23	.51	.77
26	.16	.45	.65	.85	.05	56	.61	.97	.26	.53	.80
25	.18	.47	.67	.87	.08	55	.63	99.00	.28	.56	.82
24	.20	.49	.70	.90	.11	54	.65	.02	.30	.58	.84
23	.22	.52	.72	.92	.13	53	.67	.04	.32	.60	.87
22	.25	.54	.74	.95	.16	52	.69	.06	.34	.62	.89
21	.27	.56	.77	.97	.18	51	.71	.08	.36	.65	.92
20	.29	.59	.79	96.00	.21	50	.73	.10	.39	.67	.94
19	.31	.61	.82	.02	.23	49	.75	.12	.41	.69	.96
18	.33	.63	.84	.05	.26	48	.77	.14	.43	.71	.99
17	.35	.66	.86	.07	.28	47	.79	.16	.45	.74	97.01
16	.38	.68	.89	.10	.31	46	.81	.18	.47	.76	.04
15	.40	.70	.91	.12	.34	45	.83	.20	.49	.78	.06
14	.42	.72	.94	.15	.36	44	.85	.22	.51	.80	.08
13	.44	.75	.96	.17	.39	43	.87	.24	.54	.83	.11
12	.46	.77	.98	.20	.41	42	.89	.26	.56	.85	.13
11	.48	.79	97.01	.22	.44	41	.91	.28	.58	.87	.15
10	.50	.81	.03	.25	.46	40	.93	.30	.60	.89	.18
09	.53	.84	.05	.27	.49	39	.95	.32	.62	.92	.20
08	.55	.86	.08	.29	.52	38	.97	.34	.64	.94	.22
07	.57	.88	.10	.32	.54	37	.99	.36	.66	.96	.25
06	.59	.90	.12	.34	.57	36	00.00	.38	.68	.98	.27
05	.61	.92	.15	.37	.59	35	.40	.70	98.01	.29	.29
04	.63	.95	.17	.39	.62	34	.42	.73	.03	.32	.32
03	.65	.97	.19	.42	.64	33	.44	.75	.05	.34	.34
02	.67	.99	.22	.44	.67	32	.46	.77	.07	.36	.36
01	.70	98.01	.24	.47	.69	31	.48	.79	.09	.39	.39
00	.72	.03	.26	.49	.72	30	.50	.81	.12	.41	.41
0.7999	.74	.06	.29	.51	.74	29	.52	.83	.14	.43	.43
98	.76	.08	.31	.54	.77	28	.54	.85	.16	.46	.46
97	.78	.10	.33	.56	.79	27	.56	.87	.18	.48	.48
96	.80	.12	.36	.59	.82	26	.58	.89	.20	.50	.50
95	.82	.14	.38	.61	.84	25	.60	.91	.23	.52	.52
94	.84	.17	.40	.63	.87	24	.62	.93	.25	.55	.55
93	.86	.19	.43	.66	.89	23	.64	.96	.27	.57	.57
92	.88	.21	.45	.68	.92	22	.66	.98	.29	.59	.59
91	.90	.23	.47	.71	.94	21	.68	99.00	.31	.62	.62
90	.92	.26	.50	.73	.97	20	.70	.02	.33	.64	.64
89	.95	.28	.52	.75	.99	19	.72	.04	.36	.66	.66
88	.97	.30	.54	.78	96.02	18	.74	.06	.38	.68	.68
87	.99	.32	.57	.80	.04	17	.76	.08	.40	.71	.71
86	99.01	.34	.59	.83	.07	16	.78	.10	.42	.73	.73
85	.03	.36	.61	.85	.09	15	.80	.12	.44	.75	.75
84	.05	.39	.63	.87	.12	14	.82	.14	.46	.77	.77
83	.07	.41	.66	.90	.14	13	.84	.16	.48	.80	.80
82	.09	.43	.68	.92	.16	12	.86	.18	.51	.82	.82
81	.11	.45	.70	.95	.19	11	.88	.20	.53	.84	.84
80	.13	.47	.72	.97	.21	10	.90	.22	.55	.86	.86
79	.15	.49	.75	97.00	.24	09	.92	.24	.57	.89	.89
78	.17	.51	.77	.02	.26	08	.94	.27	.59	.91	.91
77	.19	.54	.79	.04	.29	07	.96	.29	.61	.93	.93
76	.21	.56	.81	.07	.31	06	.98	.31	.63	.95	.95
75	.23	.58	.84	.09	.34	05	100.00	.33	.66	.98	.98
74	.25	.60	.86	.11	.36	04	.35	.68	98.00	.98	.98
73	.27	.62	.88	.14	.38	03	.37	.70	.70	.02	.02
72	.29	.64	.90	.16	.41	02	.39	.72	.72	.04	.04
71	.31	.66	.93	.18	.43	01	.41	.74	.74	.07	.07
70	.33	.68	.95	.21	.46	00	.43	.76	.76	.09	.09

19 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Concluded.

APPARENT SPECIFIC GRAVITY	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	35/35
0.7900	99.43	98.76	98.09	0.7830	99.56
.7899	.45	.78	.11	29	.58
98	.47	.80	.13	28	.60
97	.49	.82	.15	27	.62
96	.51	.84	.18	26	.64
95	.53	.87	.20	25	.66
94	.55	.89	.22	24	.68
93	.57	.91	.24	23	.70
92	.59	.93	.26	22	.72
91	.61	.95	.29	21	.74
90	.63	.97	.31	20	.76
89	.65	.99	.33	19	.78
88	.67	99.01	.35	18	.80
87	.69	.03	.37	17	.82
86	.71	.05	.40	16	.84
85	.73	.07	.42	15	.86
84	.75	.09	.44	14	.88
83	.77	.12	.46	13	.90
82	.79	.14	.48	12	.92
81	.81	.16	.50	11	.94
80	.85	.18	.53	09	.08
79	.85	.20	.55	08	100.00
78	.86	.22	.57		
77	.88	.24	.59		
76	.90	.26	.61		
75	.92	.28	.63		
74	.94	.30	.65		
73	.96	.32	.67		
72	.98	.34	.70		
71	100.00	.36	.72		
70		.38	.74		
69		.40	.76		
68		.42	.78		
67		.44	.80		
66		.46	.82		
65		.48	.84		
64		.50	.86		
63		.52	.89		
62		.54	.91		
61		.56	.93		
60		.58	.95		
59		.60	.97		
58		.62	.99		
57		.64	99.01		
56		.66	.03		
55		.68	.05		
54		.70	.07		
53		.72	.09		
52		.74	.12		
51		.76	.14		
50		.78	.16		
49		.80	.18		
48		.72	.20		
47		.84	.22		
46		.86	.24		
45		.88	.26		
44		.90	.28		
43		.92	.30		
42		.94	.32		
41		.96	.34		
40		.98	.36		
39		100.00	.38		
38			.40		
37			.42		
36			.44		
35			.46		
34			.48		
33			.50		
32			.52		
31			.54		
30			.56		

Alcohol table for calculating the percentages of alcohol by volume at 15.56°C in 20 mixtures of ethyl alcohol and water from their Zeiss immersion refractometer readings and indices of refraction at 17.5–25°C.<sup>1</sup>

SCALE READING <sup>2</sup>	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
13.2	1.33250	....	....	....	....	....	....	....	....	0.00
13.4	3257	....	....	....	....	....	....	....	....	0.18
13.6	3265	....	....	....	....	....	....	....	0.14	0.35
13.8	3273	....	....	....	....	....	....	0.10	0.31	0.53
14.0	3281	....	....	....	....	....	0.08	0.28	0.49	0.70
14.2	3288	....	....	....	....	0.04	0.24	0.45	0.67	0.88
14.4	3296	....	....	....	....	0.21	0.41	0.63	0.84	1.06
14.6	3304	....	....	....	0.16	0.38	0.59	0.80	1.02	1.24
14.8	3312	....	....	0.14	0.34	0.55	0.77	0.98	1.19	1.40
15.0	3319	0.00	0.10	0.31	0.52	0.73	0.94	1.16	1.36	1.55
15.2	3327	0.17	0.27	0.48	0.69	0.91	1.12	1.32	1.51	1.71
15.4	3335	0.34	0.44	0.65	0.85	1.07	1.29	1.47	1.66	1.86
15.6	3343	0.51	0.60	0.82	1.03	1.24	1.44	1.62	1.82	2.01
15.8	3350	0.68	0.78	0.99	1.21	1.40	1.60	1.77	1.97	2.17
16.0	3358	0.84	0.94	1.17	1.36	1.55	1.75	1.92	2.12	2.33
16.2	3366	1.02	1.12	1.32	1.51	1.70	1.90	2.08	2.27	2.48
16.4	3374	1.18	1.29	1.47	1.66	1.85	2.05	2.24	2.43	2.62
16.6	3381	1.34	1.43	1.62	1.81	2.00	2.20	2.39	2.57	2.77
16.8	3389	1.49	1.57	1.77	1.96	2.15	2.35	2.53	2.72	2.92
17.0	3397	1.63	1.72	1.92	2.11	2.30	2.50	2.69	2.87	3.06
17.2	3405	1.77	1.87	2.06	2.26	2.45	2.65	2.82	3.02	3.21
17.4	3412	1.92	2.01	2.21	2.41	2.59	2.79	2.97	3.17	3.36
17.6	3420	2.07	2.16	2.36	2.56	2.74	2.94	3.12	3.32	3.51
17.8	3428	2.21	2.31	2.51	2.70	2.89	3.09	3.27	3.46	3.66
18.0	3435	2.36	2.45	2.66	2.85	3.04	3.23	3.42	3.61	3.81
18.2	3443	2.50	2.60	2.81	3.00	3.19	3.37	3.57	3.76	3.96
18.4	3451	2.65	2.75	2.96	3.15	3.34	3.52	3.71	3.91	4.11
18.6	3459	2.80	2.90	3.10	3.30	3.48	3.66	3.86	4.06	4.26
18.8	3466	2.95	3.05	3.25	3.45	3.63	3.81	4.01	4.21	4.41
19.0	3474	3.10	3.19	3.40	3.59	3.77	3.96	4.16	4.36	4.56
19.2	3482	3.25	3.34	3.55	3.73	3.92	4.11	4.31	4.51	4.70
19.4	3489	3.39	3.48	3.70	3.88	4.07	4.26	4.46	4.65	4.85
19.6	3497	3.53	3.63	3.84	4.03	4.22	4.41	4.61	4.80	5.00
19.8	3505	3.68	3.78	3.98	4.17	4.37	4.56	4.75	4.95	5.15
20.0	3513	3.83	3.93	4.13	4.32	4.52	4.72	4.90	5.10	5.29
20.2	3520	3.97	4.07	4.27	4.47	4.66	4.87	5.05	5.24	5.44
20.4	3528	4.12	4.22	4.42	4.61	4.82	5.01	5.20	5.38	5.58
20.6	3536	4.26	4.36	4.56	4.75	4.96	5.15	5.34	5.52	5.72
20.8	3543	4.41	4.51	4.70	4.90	5.10	5.29	5.48	5.67	5.87
21.0	3551	4.56	4.65	4.85	5.04	5.24	5.44	5.62	5.82	6.02
21.2	3559	4.70	4.80	4.99	5.19	5.39	5.58	5.77	5.96	6.16
21.4	3566	4.84	4.94	5.14	5.33	5.53	5.72	5.91	6.11	6.30
21.6	3574	4.99	5.09	5.28	5.47	5.67	5.87	6.06	6.25	6.44
21.8	3582	5.13	5.23	5.43	5.61	5.82	6.01	6.20	6.39	6.59

<sup>1</sup> Rearranged from the table of B. H. St. John, which is based upon the data of Doroshevskii and Dvorzhanchik, *J. Russ. Phys. Chem. Soc.*, 40, 101 (1908). The scale readings were converted into refractive indices by using the formula  $n_D = 1.327338 + 0.00039347X + 0.00000020446X^2$ .

<sup>2</sup> The scale readings refer only to the scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168, 1899. According to this scale, 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If the immersion refractometer used is calibrated to another arbitrary scale, the readings must be converted into refractive indices before the table is used to determine the percentage of alcohol.

20

Alcohol table.—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
22.0	1.33590	5.27	5.37	5.57	5.76	5.96	6.15	6.34	6.54	6.73
22.2	3597	5.41	5.51	5.71	5.90	6.11	6.29	6.49	6.68	6.87
22.4	3605	5.56	5.65	5.85	6.05	6.25	6.43	6.63	6.82	7.01
22.6	3613	5.70	5.80	6.00	6.19	6.39	6.57	6.77	6.96	7.16
22.8	3620	5.85	5.94	6.14	6.33	6.53	6.71	6.91	7.10	7.31
23.0	3628	5.99	6.08	6.28	6.47	6.67	6.86	7.06	7.24	7.45
23.2	3636	6.13	6.22	6.42	6.61	6.81	7.00	7.20	7.39	7.59
23.4	3643	6.27	6.36	6.56	6.75	6.95	7.14	7.34	7.53	7.73
23.6	3651	6.41	6.50	6.70	6.90	7.09	7.28	7.48	7.67	7.87
23.8	3659	6.55	6.64	6.85	7.04	7.23	7.42	7.62	7.81	8.00
24.0	3666	6.69	6.78	6.99	7.18	7.38	7.56	7.76	7.95	8.14
24.2	3674	6.83	6.92	7.13	7.32	7.52	7.70	7.90	8.09	8.28
24.4	3682	6.97	7.06	7.27	7.46	7.66	7.84	8.04	8.23	8.42
24.6	3689	7.11	7.20	7.41	7.60	7.80	7.98	8.17	8.37	8.55
24.8	3697	7.25	7.35	7.55	7.74	7.93	8.12	8.31	8.51	8.69
25.0	3705	7.39	7.49	7.68	7.88	8.06	8.26	8.45	8.64	8.84
25.2	3712	7.53	7.63	7.82	8.01	8.20	8.40	8.59	8.78	8.98
25.4	3720	7.66	7.76	7.95	8.14	8.34	8.54	8.73	8.92	9.12
25.6	3728	7.80	7.90	8.09	8.28	8.48	8.68	8.86	9.06	9.26
25.8	3735	7.94	8.03	8.22	8.42	8.62	8.82	9.00	9.20	9.39
26.0	3743	8.07	8.16	8.36	8.55	8.75	8.95	9.14	9.34	9.53
26.2	3751	8.21	8.30	8.50	8.69	8.89	9.09	9.28	9.48	9.67
26.4	3758	8.34	8.44	8.63	8.82	9.03	9.22	9.42	9.61	9.81
26.6	3766	8.48	8.57	8.77	8.96	9.16	9.36	9.55	9.75	9.95
26.8	3774	8.62	8.71	8.91	9.10	9.30	9.49	9.69	9.89	10.09
27.0	3781	8.75	8.85	9.05	9.23	9.44	9.63	9.83	10.03	10.23
27.2	3789	8.89	8.98	9.18	9.37	9.58	9.76	9.97	10.17	10.37
27.4	3796	9.02	9.12	9.32	9.51	9.71	9.90	10.10	10.31	10.51
27.6	3804	9.16	9.26	9.45	9.65	9.85	10.03	10.24	10.45	10.65
27.8	3812	9.29	9.39	9.59	9.79	9.98	10.17	10.38	10.58	10.79
28.0	3820	9.43	9.53	9.72	9.92	10.12	10.31	10.51	10.72	10.93
28.2	3827	9.57	9.66	9.86	10.06	10.25	10.45	10.65	10.86	11.06
28.4	3835	9.70	9.80	9.99	10.19	10.39	10.59	10.79	11.00	11.20
28.6	3842	9.84	9.93	10.13	10.32	10.52	10.72	10.93	11.13	11.33
28.8	3850	9.97	10.07	10.26	10.46	10.66	10.86	11.06	11.27	11.47
29.0	3858	10.10	10.19	10.40	10.59	10.79	11.00	11.20	11.40	11.61
29.2	3865	10.24	10.33	10.52	10.73	10.93	11.13	11.33	11.54	11.75
29.4	3873	10.36	10.46	10.66	10.86	11.06	11.27	11.47	11.67	11.88
29.6	3881	10.50	10.59	10.79	10.99	11.20	11.39	11.60	11.81	12.01
29.8	3888	10.63	10.72	10.93	11.12	11.33	11.53	11.74	11.94	12.15
30.0	3896	10.76	10.86	11.05	11.26	11.46	11.66	11.87	12.08	12.29
30.2	3904	10.89	10.99	11.18	11.38	11.59	11.79	12.00	12.21	12.42
30.4	3911	11.02	11.12	11.31	11.51	11.72	11.93	12.13	12.34	12.56
30.6	3919	11.15	11.25	11.44	11.64	11.85	12.06	12.27	12.48	12.70
30.8	3926	11.28	11.38	11.58	11.78	11.99	12.19	12.40	12.61	12.84



*Alcohol table.*—Continued.

SCALE. READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
31.0	1.33934	11.41	11.51	11.71	11.91	12.12	12.32	12.54	12.75	12.97
31.2	3942	11.54	11.64	11.84	12.04	12.25	12.46	12.67	12.89	13.11
31.4	3949	11.66	11.77	11.97	12.17	12.38	12.59	12.81	13.02	13.24
31.6	3957	11.79	11.90	12.10	12.30	12.51	12.72	12.94	13.15	13.37
31.8	3964	11.92	12.03	12.23	12.43	12.64	12.85	13.07	13.29	13.51
32.0	3972	12.05	12.15	12.36	12.57	12.78	12.99	13.20	13.42	13.64
32.2	3980	12.18	12.28	12.49	12.70	12.91	13.12	13.34	13.55	13.77
32.4	3987	12.31	12.40	12.62	12.83	13.04	13.25	13.47	13.69	13.91
32.6	3995	12.43	12.54	12.75	12.96	13.17	13.38	13.60	13.82	14.04
32.8	4002	12.56	12.67	12.88	13.09	13.30	13.51	13.73	13.95	14.17
33.0	4010	12.69	12.79	13.01	13.22	13.43	13.64	13.86	14.09	14.31
33.2	4018	12.82	12.92	13.13	13.35	13.56	13.78	13.99	14.22	14.44
33.4	4025	12.95	13.05	13.26	13.48	13.69	13.91	14.13	14.35	14.58
33.6	4033	13.08	13.18	13.39	13.61	13.82	14.04	14.26	14.48	14.71
33.8	4040	13.20	13.30	13.52	13.74	13.95	14.17	14.39	14.62	14.85
34.0	4048	13.33	13.43	13.64	13.86	14.08	14.30	14.52	14.75	14.98
34.2	4056	13.45	13.56	13.77	13.99	14.21	14.43	14.65	14.88	15.11
34.4	4063	13.58	13.68	13.90	14.12	14.34	14.57	14.78	15.01	15.25
34.6	4071	13.70	13.81	14.02	14.25	14.47	14.70	14.91	15.14	15.38
34.8	4078	13.83	13.94	14.14	14.37	14.59	14.83	15.05	15.28	15.51
35.0	4086	13.96	14.06	14.27	14.50	14.72	14.96	15.18	15.41	15.65
35.2	4094	14.08	14.19	14.39	14.62	14.85	15.09	15.31	15.54	15.78
35.4	4101	14.21	14.31	14.52	14.75	14.97	15.22	15.44	15.67	15.91
35.6	4109	14.33	14.44	14.65	14.87	15.10	15.34	15.56	15.80	16.05
35.8	4116	14.46	14.56	14.78	15.00	15.23	15.47	15.69	15.93	16.18
36.0	4124	14.58	14.69	14.90	15.13	15.35	15.59	15.82	16.06	16.31
36.2	4131	14.71	14.81	15.03	15.25	15.48	15.72	15.95	16.19	16.44
36.4	4139	14.83	14.94	15.16	15.38	15.61	15.85	16.08	16.32	16.56
36.6	4146	14.96	15.06	15.28	15.51	15.73	15.97	16.21	16.45	16.69
36.8	4154	15.08	15.19	15.41	15.63	15.86	16.10	16.34	16.58	16.82
37.0	4162	15.20	15.31	15.53	15.76	15.99	16.23	16.47	16.71	16.95
37.2	4169	15.33	15.44	15.66	15.89	16.11	16.35	16.60	16.84	17.08
37.4	4177	15.45	15.56	15.79	16.01	16.24	16.48	16.72	16.97	17.21
37.6	4184	15.57	15.69	15.91	16.14	16.37	16.61	16.85	17.09	17.34
37.8	4192	15.70	15.81	16.04	16.26	16.49	16.73	16.98	17.22	17.46
38.0	4199	15.82	15.94	16.16	16.39	16.62	16.86	17.11	17.35	17.59
38.2	4207	15.94	16.06	16.29	16.51	16.75	16.99	17.23	17.47	17.72
38.4	4215	16.07	16.18	16.41	16.64	16.87	17.11	17.36	17.60	17.85
38.6	4222	16.19	16.31	16.53	16.76	17.00	17.24	17.48	17.73	17.97
38.8	4230	16.31	16.43	16.66	16.89	17.13	17.36	17.61	17.85	18.10
39.0	4237	16.44	16.55	16.78	17.01	17.25	17.49	17.74	17.98	18.23
39.2	4245	16.56	16.67	16.91	17.14	17.38	17.62	17.86	18.11	18.35
39.4	4252	16.68	16.80	17.03	17.26	17.50	17.74	17.99	18.23	18.48
39.6	4260	16.80	16.92	17.15	17.39	17.63	17.87	18.11	18.36	18.61
39.8	4267	16.93	17.04	17.28	17.51	17.75	17.99	18.24	18.48	18.73

20

*Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
40.0	1.34275	17.05	17.16	17.40	17.63	17.88	18.12	18.36	18.61	18.86
40.2	4282	17.17	17.29	17.52	17.76	18.00	18.24	18.49	18.74	18.99
40.4	4290	17.29	17.41	17.64	17.88	18.12	18.37	18.61	18.86	19.11
40.6	4298	17.41	17.53	17.77	18.01	18.25	18.49	18.74	18.99	19.24
40.8	4305	17.54	17.65	17.89	18.13	18.37	18.61	18.86	19.11	19.37
41.0	4313	17.66	17.77	18.01	18.25	18.49	18.74	18.99	19.24	19.49
41.2	4320	17.78	17.90	18.13	18.37	18.62	18.86	19.11	19.36	19.62
41.4	4328	17.90	18.03	18.26	18.50	18.74	18.99	19.24	19.49	19.75
41.6	4335	18.02	18.14	18.38	18.62	18.86	19.11	19.36	19.61	19.87
41.8	4343	18.14	18.26	18.50	18.74	18.99	19.23	19.48	19.74	20.00
42.0	4350	18.27	18.38	18.62	18.87	19.11	19.36	19.61	19.86	20.13
42.2	4358	18.39	18.50	18.74	18.99	19.23	19.48	19.73	19.99	20.25
42.4	4365	18.51	18.62	18.87	19.11	19.36	19.60	19.86	20.11	20.38
42.6	4373	18.63	18.75	18.99	19.23	19.48	19.72	19.98	20.24	20.50
42.8	4380	18.75	18.87	19.11	19.36	19.60	19.85	20.10	20.36	20.63
43.0	4388	18.87	18.99	19.23	19.48	19.72	19.97	20.23	20.49	20.75
43.2	4395	18.99	19.11	19.35	19.60	19.85	20.09	20.35	20.61	20.88
43.4	4403	19.11	19.23	19.47	19.72	19.97	20.21	20.47	20.74	21.01
43.6	4410	19.23	19.35	19.59	19.85	20.09	20.34	20.60	20.86	21.13
43.8	4418	19.35	19.47	19.72	19.97	20.21	20.46	20.72	20.99	21.25
44.0	4426	19.46	19.59	19.84	20.09	20.34	20.58	20.84	21.11	21.38
44.2	4433	19.58	19.71	19.96	20.21	20.46	20.71	20.96	21.23	21.50
44.4	4440	19.70	19.83	20.08	20.33	20.58	20.83	21.09	21.36	21.63
44.6	4448	19.82	19.95	20.20	20.45	20.70	20.95	21.21	21.48	21.75
44.8	4456	19.94	20.07	20.32	20.58	20.82	21.07	21.33	21.60	21.88
45.0	4463	20.06	20.18	20.44	20.70	20.95	21.19	21.45	21.73	22.00
45.2	4470	20.18	20.30	20.56	20.82	21.07	21.31	21.58	21.85	22.13
45.4	4478	20.29	20.42	20.68	20.94	21.19	21.43	21.70	21.98	22.25
45.6	4486	20.41	20.54	20.80	21.06	21.31	21.55	21.82	22.10	22.38
45.8	4493	20.53	20.66	20.92	21.18	21.43	21.67	21.94	22.23	22.51
46.0	4500	20.65	20.78	21.04	21.30	21.54	21.79	22.07	22.35	22.64
46.2	4508	20.76	20.89	21.16	21.42	21.66	21.91	22.19	22.48	22.76
46.4	4516	20.88	21.01	21.28	21.54	21.78	22.03	22.32	22.61	22.89
46.6	4523	21.00	21.13	21.40	21.66	21.90	22.16	22.44	22.73	23.02
46.8	4530	21.12	21.25	21.52	21.78	22.02	22.28	22.57	22.86	23.15
47.0	4538	21.24	21.37	21.64	21.90	22.15	22.41	22.69	22.99	23.28
47.2	4545	21.36	21.49	21.76	22.02	22.27	22.53	22.82	23.12	23.41
47.4	4553	21.48	21.61	21.88	22.15	22.39	22.66	22.94	23.24	23.54
47.6	4560	21.60	21.73	22.00	22.27	22.51	22.78	23.07	23.37	23.67
47.8	4568	21.72	21.85	22.12	22.39	22.64	22.91	23.20	23.50	23.80
48.0	4575	21.84	21.97	22.24	22.51	22.76	23.03	23.32	23.63	23.93
48.2	4583	21.96	22.09	22.36	22.63	22.88	23.16	23.45	23.76	24.06
48.4	4590	22.08	22.21	22.48	22.75	23.01	23.28	23.58	23.89	24.19
48.6	4598	22.20	22.33	22.60	22.87	23.13	23.41	23.71	24.02	24.32
48.8	4605	22.32	22.45	22.72	22.99	23.26	23.54	23.83	24.14	24.45

*Alcohol table.—Continued.*

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
49.0	1.34613	22.44	22.57	22.84	23.12	23.38	23.66	23.96	24.27	24.59
49.2	4620	22.56	22.69	22.96	23.24	23.51	23.79	24.09	24.40	24.72
49.4	4628	22.68	22.81	23.08	23.36	23.63	23.92	24.22	24.53	24.85
49.6	4635	22.80	22.93	23.21	23.48	23.76	24.04	24.35	24.66	24.98
49.8	4643	22.92	23.05	23.33	23.61	23.88	24.17	24.48	24.79	25.11
50.0	4650	23.04	23.17	23.45	23.73	24.01	24.30	24.61	24.92	25.25
50.2	4658	23.16	23.30	23.57	23.85	24.13	24.43	24.74	25.05	25.38
50.4	4665	23.28	23.42	23.69	23.98	24.26	24.56	24.86	25.18	25.51
50.6	4672	23.40	23.54	23.81	24.10	24.38	24.69	24.99	25.32	25.65
50.8	4680	23.51	23.66	23.93	24.22	24.51	24.81	25.12	25.45	25.78
51.0	4687	23.63	23.78	24.05	24.35	24.64	24.94	25.25	25.58	25.91
51.2	4695	23.75	23.90	24.18	24.47	24.76	25.07	25.38	25.71	26.05
51.4	4702	23.87	24.02	24.30	24.59	24.89	25.20	25.51	25.84	26.18
51.6	4710	23.99	24.14	24.42	24.72	25.01	25.33	25.64	25.97	26.32
51.8	4717	24.11	24.26	24.54	24.84	25.14	25.46	25.77	26.11	26.45
52.0	4724	24.23	24.38	24.66	24.96	25.27	25.58	25.90	26.24	26.59
52.2	4732	24.36	24.50	24.79	25.09	25.39	25.71	26.03	26.37	26.72
52.4	4740	24.48	24.62	24.91	25.21	25.52	25.84	26.16	26.51	26.86
52.6	4747	24.60	24.74	25.03	25.34	25.65	25.97	26.29	26.64	26.99
52.8	4754	24.72	24.86	25.15	25.46	25.77	26.10	26.42	26.77	27.13
53.0	4762	24.84	24.98	25.28	25.59	25.90	26.23	26.56	26.91	27.27
53.2	4769	24.96	25.10	25.40	25.71	26.03	26.35	26.69	27.04	27.40
53.4	4777	25.08	25.23	25.52	25.84	26.15	26.48	26.82	27.17	27.54
53.6	4784	25.20	25.35	25.65	25.96	26.28	26.61	26.95	27.31	27.67
53.8	4792	25.32	25.47	25.77	26.09	26.41	26.74	27.08	27.44	27.81
54.0	4799	25.44	25.59	25.90	26.22	26.54	26.87	27.21	27.58	27.95
54.2	4806	25.56	25.71	26.02	26.34	26.67	27.00	27.35	27.71	28.08
54.4	4814	25.68	25.84	26.14	26.47	26.79	27.13	27.48	27.85	28.22
54.6	4821	25.81	25.96	26.27	26.59	26.92	27.26	27.61	27.98	28.36
54.8	4829	25.93	26.08	26.39	26.72	27.05	27.39	27.75	28.11	28.49
55.0	4836	26.05	26.20	26.52	26.85	27.18	27.52	27.88	28.25	28.63
55.2	4844	26.17	26.32	26.64	26.97	27.31	27.65	28.01	28.38	28.77
55.4	4851	26.29	26.45	26.76	27.10	27.43	27.78	28.15	28.52	28.90
55.6	4858	26.41	26.57	26.89	27.23	27.55	27.92	28.28	28.65	29.04
55.8	4866	26.53	26.69	27.01	27.35	27.69	28.05	28.41	28.78	29.18
56.0	4873	26.65	26.81	27.14	27.48	27.82	28.18	28.54	28.92	29.31
56.2	4880	26.78	26.93	27.26	27.60	27.94	28.31	28.68	29.05	29.45
56.4	4888	26.90	27.05	27.38	27.73	28.07	28.44	28.81	29.19	29.58
56.6	4895	27.02	27.18	27.51	27.85	28.20	28.56	28.94	29.32	29.72
56.8	4903	27.14	27.30	27.63	27.98	28.33	28.69	29.07	29.46	29.86
57.0	4910	27.26	27.42	27.75	28.10	28.46	28.82	29.20	29.59	29.99
57.2	4918	27.38	27.54	27.88	28.23	28.59	28.95	29.34	29.73	30.13
57.4	4925	27.50	27.66	28.00	28.35	28.72	29.08	29.47	29.86	30.27
57.6	4932	27.62	27.79	28.13	28.48	28.85	29.21	29.60	30.00	30.41
57.8	4940	27.75	27.91	28.25	28.60	28.97	29.34	29.73	30.14	30.55

*Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
58.0	1.34947	27.87	28.03	28.38	28.73	29.10	29.47	29.87	30.27	30.69
58.2	4954	27.99	28.15	28.50	28.86	29.23	29.60	29.99	30.41	30.83
58.4	4962	28.11	28.28	28.62	28.98	29.36	29.73	30.13	30.54	30.97
58.6	4969	28.23	28.40	28.75	29.11	29.48	29.86	30.26	30.68	31.11
58.8	4977	28.35	28.52	28.88	29.23	29.61	29.99	30.40	30.82	31.25
59.0	4984	28.47	28.64	29.00	29.36	29.74	30.13	30.53	30.95	31.40
59.2	4991	28.59	28.77	29.12	29.49	29.87	30.26	30.67	31.09	31.54
59.4	4999	28.71	28.89	29.25	29.61	29.99	30.39	30.81	31.23	31.68
59.6	5006	28.84	29.01	29.37	29.74	30.13	30.53	30.94	31.38	31.83
59.8	5014	28.96	29.13	29.50	29.87	30.26	30.66	31.08	31.52	31.97
60.0	5021	29.08	29.26	29.62	29.99	30.39	30.79	31.22	31.66	32.12
60.2	5028	29.20	29.38	29.74	30.12	30.52	30.93	31.36	31.80	32.27
60.4	5036	29.32	29.50	29.87	30.25	30.65	31.06	31.50	31.94	32.41
60.6	5043	29.45	29.63	29.99	30.38	30.78	31.20	31.64	32.09	32.56
60.8	5050	29.57	29.75	30.12	30.51	30.91	31.33	31.78	32.23	32.71
61.0	5058	29.69	29.87	30.25	30.64	31.05	31.47	31.92	32.38	32.86
61.2	5065	29.81	29.99	30.38	30.77	31.18	31.61	32.06	32.52	33.01
61.4	5073	29.93	30.12	30.50	30.90	31.32	31.74	32.20	32.67	33.16
61.6	5080	30.06	30.25	30.63	31.03	31.45	31.88	32.34	32.81	33.31
61.8	5087	30.18	30.37	30.76	31.16	31.59	32.01	32.49	32.96	33.46
62.0	5095	30.31	30.50	30.89	31.29	31.72	32.16	32.63	33.10	33.60
62.2	5102	30.43	30.63	31.01	31.43	31.86	32.30	32.77	33.25	33.75
62.4	5110	30.56	30.75	31.14	31.56	31.99	32.44	32.91	33.40	33.90
62.6	5117	30.69	30.88	31.28	31.69	32.13	32.58	33.06	33.55	34.05
62.8	5124	30.81	31.01	31.41	31.83	32.27	32.72	33.20	33.70	34.21
63.0	5132	30.94	31.14	31.54	31.96	32.41	32.87	33.35	33.84	34.36
63.2	5139	31.06	31.26	31.67	32.10	32.55	33.01	33.50	33.99	34.52
63.4	5146	31.19	31.39	31.80	32.23	32.69	33.15	33.64	34.15	34.67
63.6	5154	31.32	31.52	31.93	32.37	32.83	33.30	33.79	34.30	34.83
63.8	5161	31.45	31.65	32.07	32.51	32.97	33.44	33.93	34.45	34.98
64.0	5168	31.58	31.78	32.20	32.65	33.11	33.59	34.08	34.61	35.15
64.2	5176	31.70	31.91	32.34	32.79	33.25	33.73	34.23	34.76	35.31
64.4	5183	31.83	32.04	32.47	32.92	33.39	33.88	34.39	34.92	35.48
64.6	5190	31.96	32.17	32.60	33.06	33.53	34.02	34.54	35.07	35.64
64.8	5198	32.09	32.30	32.74	33.20	33.67	34.17	34.69	35.23	35.80
65.0	5205	32.22	32.43	32.87	33.34	33.82	34.32	34.84	35.39	35.97
65.2	5212	32.35	32.57	33.01	33.48	33.96	34.47	34.99	35.55	36.13
65.4	5220	32.48	32.70	33.15	33.62	34.10	34.61	35.15	35.71	36.30
65.6	5227	32.61	32.83	33.28	33.76	34.25	34.76	35.30	35.87	36.46
65.8	5234	32.75	32.96	33.42	33.90	34.40	34.91	35.46	36.02	36.63
66.0	5242	32.88	33.10	33.56	34.04	34.54	35.06	35.62	36.19	36.79
66.2	5249	33.01	33.23	33.70	34.18	34.69	35.22	35.77	36.35	36.96
66.4	5256	33.14	33.37	33.84	34.33	34.84	35.38	35.93	36.52	37.13
66.6	5264	33.28	33.51	33.98	34.47	34.99	35.53	36.09	36.68	37.30
66.8	5271	33.41	33.65	34.12	34.62	35.14	35.69	36.25	36.84	37.48

*Alcohol table.—Continued.*

20

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
67.0	1.35278	33.55	33.79	34.26	34.76	35.29	35.84	36.41	37.01	37.65
67.2	5286	33.69	33.92	34.41	34.91	35.44	36.00	36.57	37.18	37.83
67.4	5293	33.82	34.06	34.55	35.05	35.60	36.16	36.73	37.35	38.00
67.6	5300	33.96	34.20	34.69	35.20	35.75	36.32	36.90	37.52	38.18
67.8	5308	34.09	34.34	34.84	35.35	35.90	36.48	37.06	37.69	38.35
68.0	5315	34.23	34.48	34.98	35.50	36.05	36.63	37.23	37.86	38.53
68.2	5322	34.36	34.62	35.13	35.65	36.21	36.79	37.39	38.03	38.70
68.4	5329	34.50	34.76	35.27	35.80	36.37	36.95	37.56	38.21	38.88
68.6	5337	34.64	34.90	35.42	35.95	36.52	37.12	37.73	38.38	39.06
68.8	5344	34.77	35.04	35.57	36.10	36.68	37.28	37.90	38.56	39.24
69.0	5351	34.91	35.19	35.71	36.25	36.84	37.45	38.07	38.73	39.43
69.2	5359	35.04	35.33	35.86	36.41	36.99	37.61	38.24	38.90	39.61
69.4	5366	35.19	35.47	36.01	36.56	37.15	37.78	38.41	39.08	39.80
69.6	5373	35.34	35.62	36.16	36.72	37.32	37.94	38.58	39.26	39.98
69.8	5381	35.49	35.76	36.31	36.87	37.48	38.11	38.75	39.45	40.17
70.0	5388	35.64	35.91	36.46	37.02	37.64	38.28	38.92	39.63	40.35
70.2	5395	35.78	36.05	36.61	37.19	37.80	38.45	39.10	39.81	40.53
70.4	5402	35.93	36.20	36.76	37.35	37.97	38.61	39.28	39.99	40.72
70.6	5410	36.08	36.35	36.92	37.51	38.13	38.78	39.46	40.17	40.90
70.8	5417	36.23	36.50	37.07	37.67	38.30	38.95	39.64	40.35	41.08
71.0	5424	36.38	36.65	37.23	37.83	38.47	39.12	39.82	40.54	41.27
71.2	5432	36.53	36.80	37.39	37.99	38.63	39.30	40.00	40.72	41.46
71.4	5439	36.68	36.95	37.55	38.16	38.80	39.48	40.18	40.90	41.64
71.6	5446	36.83	37.11	37.71	38.32	38.97	39.65	40.36	41.08	41.83
71.8	5454	36.98	37.27	37.87	38.49	39.14	39.83	40.54	41.27	42.02
72.0	5461	37.13	37.42	38.02	38.65	39.31	40.01	40.72	41.45	42.21
72.2	5468	37.29	37.58	38.19	38.82	39.49	40.18	40.90	41.64	42.40
72.4	5475	37.44	37.73	38.35	38.98	39.66	40.36	41.08	41.82	42.58
72.6	5483	37.60	37.89	38.51	39.16	39.83	40.54	41.26	42.01	42.77
72.8	5490	37.75	38.05	38.67	39.33	40.01	40.71	41.45	42.19	42.96
73.0	5497	37.91	38.21	38.84	39.50	40.18	40.88	41.63	42.38	43.15
73.2	5504	38.06	38.37	39.00	39.67	40.36	41.06	41.81	42.56	43.33
73.4	5512	38.22	38.53	39.17	39.84	40.53	41.24	41.99	42.75	43.52
73.6	5519	38.38	38.69	39.34	40.02	40.70	41.42	42.17	42.93	43.70
73.8	5526	38.54	38.85	39.50	40.19	40.88	41.60	42.36	43.12	43.89
74.0	5533	38.70	39.01	39.67	40.36	41.05	41.78	42.54	43.31	44.08
74.2	5541	38.86	39.18	39.84	40.53	41.23	41.96	42.72	43.49	44.28
74.4	5548	39.02	39.34	40.01	40.71	41.41	42.15	42.91	43.68	44.48
74.6	5555	39.18	39.51	40.18	40.88	41.59	42.33	43.09	43.86	44.67
74.8	5563	39.35	39.68	40.35	41.05	41.77	42.51	43.28	44.05	44.87
75.0	5570	39.51	39.84	40.53	41.23	41.95	42.70	43.46	44.25	45.07
75.2	5577	39.68	40.01	40.70	41.41	42.13	42.88	43.65	44.44	45.29
75.4	5584	39.84	40.18	40.87	41.58	42.31	43.07	43.83	44.63	45.50
75.6	5592	40.01	40.35	41.04	41.76	42.49	43.25	44.02	44.83	45.71
75.8	5599	40.18	40.53	41.22	41.94	42.67	43.44	44.21	45.03	45.92

*Alcohol table.—Concluded.*

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
76.0	1.35606	40.35	40.70	41.40	42.12	42.85	43.63	44.41	45.24	46.12
76.2	5613	40.53	40.87	41.57	42.30	43.04	43.81	44.60	45.44	46.34
76.4	5621	40.70	41.04	41.75	42.48	43.22	44.00	44.80	45.65	46.56
76.6	5628	40.87	41.22	41.92	42.66	43.41	44.19	44.99	45.86	46.78
76.8	5635	41.04	41.39	42.10	42.84	43.60	44.38	45.19	46.07	47.00
77.0	5642	41.22	41.57	42.28	43.02	43.79	44.57	45.40	46.29	47.23
77.2	5650	41.39	41.74	42.46	43.20	43.97	44.76	45.60	46.51	47.45
77.4	5657	41.57	41.91	42.63	43.39	44.16	44.95	45.81	46.73	47.68
77.6	5664	41.75	42.09	42.81	43.57	44.35	45.15	46.01	46.95	47.91
77.8	5671	41.92	42.26	42.99	43.76	44.54	45.35	46.23	47.17	48.14
78.0	5678	42.09	42.43	43.17	43.94	44.73	45.56	46.45	47.40	48.37
78.2	5686	42.26	42.61	43.36	44.13	44.92	45.76	46.67	47.63	48.60
78.4	5693	42.44	42.78	43.54	44.32	45.12	45.96	46.89	47.85	48.84
78.6	5700	42.61	42.96	43.72	44.51	45.32	46.17	47.11	48.08	49.07
78.8	5707	42.78	43.14	43.91	44.70	45.52	46.39	47.34	48.31	49.31
79.0	5715	42.95	43.32	44.09	44.89	45.72	46.61	47.56	48.53	49.54
79.2	5722	43.13	43.50	44.28	45.08	45.92	46.83	47.79	48.76	49.77
79.4	5729	43.31	43.68	44.47	45.28	46.13	47.04	48.01	48.99	50.01
79.6	5736	43.49	43.86	44.65	45.48	46.34	47.26	48.23	49.22	50.24
79.8	5744	43.67	44.05	44.84	45.68	46.56	47.48	48.46	49.45	50.48
80.0	5751	43.85	44.24	45.04	45.88	46.77	47.70	48.68	49.68	50.71

Percentages by weight corresponding to various percentages by volume at 21  
15.56°C. (60°F.) in mixtures of ethyl alcohol and water.<sup>1</sup>

PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES	PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES
0	0.000	.....	50	42.487	.....
1	0.795	0.795	51	43.428	0.941
2	1.593	.798	52	44.374	.946
3	2.392	.799	53	45.326	.952
4	3.194	.802	54	46.283	.957
		.804			.962
5	3.998		55	47.245	
6	4.804	.806	56	48.214	.969
7	5.612	.808	57	49.187	.973
8	6.422	.810	58	50.167	.980
9	7.234	.812	59	51.154	.987
		.813			.993
10	8.047		60	52.147	
11	8.862	.815	61	53.146	.999
12	9.679	.817	62	54.152	1.006
13	10.497	.818	63	55.165	1.013
14	11.317	.820	64	56.184	1.019
		.821			1.024
15	12.138		65	57.208	
16	12.961	.823	66	58.241	1.033
17	13.786	.825	67	59.279	1.038
18	14.612	.826	68	60.325	1.046
19	15.440	.828	69	61.379	1.054
		.829			1.062
20	16.269		70	62.441	
21	17.100	.831	71	63.511	1.070
22	17.933	.833	72	64.588	1.077
23	18.768	.835	73	65.674	1.086
24	19.604	.836	74	66.768	1.094
		.839			1.102
25	20.443		75	67.870	
26	21.285	.842	76	68.982	1.112
27	22.127	.842	77	70.102	1.120
28	22.973	.846	78	71.234	1.132
29	23.820	.847	79	72.375	1.141
		.850			1.151
30	24.670		80	73.526	
31	25.524	.854	81	74.686	1.160
32	26.382	.858	82	75.858	1.172
33	27.242	.860	83	77.039	1.181
34	28.104	.862	84	78.233	1.194
		.867			1.208
35	28.971		85	79.441	
36	29.842	.871	86	80.662	1.221
37	30.717	.875	87	81.897	1.235
38	31.596	.879	88	83.144	1.247
39	32.478	.882	89	84.408	1.264
		.886			1.281
40	33.364		90	85.689	
41	34.254	.890	91	86.989	1.300
42	35.150	.896	92	88.310	1.321
43	36.050	.900	93	89.652	1.342
44	36.955	.905	94	91.025	1.373
		.910			1.398
45	37.865		95	92.423	
46	38.778	.913	96	93.851	1.428
47	39.697	.919	97	95.315	1.464
48	40.622	.925	98	96.820	1.505
49	41.551	.929	99	98.381	1.561
		.936			1.619
50	42.487	.....	100	100.000	.....

<sup>1</sup> Bureau of Standards Circular No. 19, p. 18 (1924).

22 For determining added water in milk by means of the freezing-point depression  
(based on *Winter's table*<sup>1</sup>).

(For practical purposes the added water results may be expressed to the nearest decimal.)

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.550	0.00	0.505	8.18	0.460	16.36
.549	0.18	.504	8.36	.459	16.54
.548	0.36	.503	8.54	.458	16.73
.547	0.54	.502	8.73	.457	16.91
.546	0.73	.501	8.91	.456	17.09
.545	0.91	.500	9.09	.455	17.27
.544	1.09	.499	9.27	.454	17.45
.543	1.27	.498	9.45	.453	17.64
.542	1.45	.497	9.64	.452	17.82
.541	1.63	.496	9.82	.451	18.00
.540	1.82	.495	10.00	.450	18.18
.539	2.00	.494	10.18	.449	18.36
.538	2.18	.493	10.36	.448	18.54
.537	2.36	.492	10.54	.447	18.73
.536	2.54	.491	10.72	.446	18.91
.535	2.72	.490	10.91	.445	19.09
.534	2.91	.489	11.09	.444	19.27
.533	3.09	.488	11.27	.443	19.45
.532	3.27	.487	11.45	.442	19.64
.531	3.45	.486	11.64	.441	19.82
.530	3.64	.485	11.82	.440	20.00
.529	3.82	.484	12.00	.439	20.18
.528	4.00	.483	12.18	.438	20.36
.527	4.18	.482	12.36	.437	20.54
.526	4.36	.481	12.54	.436	20.73
.525	4.54	.480	12.73	.435	20.91
.524	4.73	.479	12.91	.434	21.09
.523	4.91	.478	13.09	.433	21.27
.522	5.09	.477	13.27	.432	21.45
.521	5.27	.476	13.45	.431	21.64
.520	5.45	.475	13.64	.430	21.82
.519	5.63	.474	13.82	.429	22.00
.518	5.82	.473	14.00	.428	22.18
.517	6.00	.472	14.18	.427	22.36
.516	6.18	.471	14.37	.426	22.54
.515	6.36	.470	14.54	.425	22.73
.514	6.54	.469	14.73	.424	22.91
.513	6.73	.468	14.91	.423	23.09
.512	6.91	.467	15.09	.422	23.27
.511	7.09	.466	15.27	.421	23.45
.510	7.27	.465	15.45	.420	23.64
.509	7.45	.464	15.63	.419	23.82
.508	7.64	.463	15.82	.418	24.00
.507	7.82	.462	16.00	.417	24.18
.506	8.00	.461	16.18	.416	24.36

<sup>1</sup> *Chem. News*, 110, 283 (1914).



*For determining added water in milk by means of the freezing-point depression 22*  
(based on Winter's table).—Concluded.

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.415	24.54	0.390	29.09	0.365	33.64
.414	24.73	.389	29.27	.364	33.82
.413	24.91	.388	29.45	.363	34.00
.412	25.09	.387	29.64	.362	34.18
.411	25.27	.386	29.82	.361	34.36
.410	25.45	.385	30.00	.360	34.54
.409	25.64	.384	30.18	.359	34.73
.408	25.82	.383	30.36	.358	34.91
.407	26.00	.382	30.54	.357	35.09
.406	26.18	.381	30.73	.356	35.27
.405	26.36	.380	30.91	.355	35.45
.404	26.54	.379	31.09	.354	35.64
.403	26.73	.378	31.27	.353	35.82
.402	26.91	.377	31.45	.352	36.00
.401	27.09	.376	31.64	.351	36.18
.400	27.27	.375	31.82	.350	36.36
.399	27.45	.374	32.00		
.398	27.64	.373	32.18		
.397	27.82	.372	32.36		
.396	28.00	.371	32.54		
.395	28.18	.370	32.73		
.394	28.36	.369	32.91		
.393	28.54	.368	33.09		
.392	28.73	.367	33.27		
.391	28.91	.366	33.45		

23

*Density of carbon dioxide (Parr).<sup>1</sup>*

(Weight in milligrams of 1 ml of carbon dioxide at 700–770 mm pressure and 10–30°C. Corrected for aqueous vapor and barometer readings on glass scale. Calculated from 1.976 equals weight of liter of CO<sub>2</sub> at 0°C., 760 mm pressure and 41° latitude.)

mm.	10°	11°	12°	13°	14°	15°	16°	17°	18°	19°
700	1.7288	1.7201	1.7113	1.7020	1.6927	1.6863	1.6799	1.6716	1.6632	1.6547
702	.7338	.7252	.7164	.7072	.6980	.6914	.6848	.6765	.6680	.6595
704	.7388	.7302	.7215	.7124	.7033	.6965	.6897	.6813	.6729	.6644
706	.7438	.7353	.7266	.7176	.7086	.7016	.6946	.6862	.6778	.6692
708	.7488	.7403	.7317	.7228	.7139	.7067	.6995	.6911	.6826	.6741
710	.7538	.7453	.7368	.7280	.7192	.7118	.7044	.6960	.6874	.6789
712	.7588	.7504	.7419	.7332	.7245	.7169	.7092	.7008	.6922	.6837
714	.7638	.7555	.7470	.7384	.7298	.7220	.7141	.7057	.6970	.6886
716	.7688	.7605	.7521	.7436	.7351	.7271	.7190	.7106	.7019	.6934
718	.7738	.7656	.7572	.7488	.7404	.7322	.7239	.7154	.7068	.6983
720	.7788	.7706	.7623	.7540	.7457	.7373	.7288	.7203	.7117	.7031
722	.7838	.7756	.7673	.7590	.7506	.7422	.7337	.7252	.7166	.7079
724	.7888	.7806	.7723	.7639	.7555	.7471	.7386	.7301	.7215	.7128
726	.7938	.7856	.7773	.7689	.7605	.7520	.7435	.7349	.7263	.7176
728	.7988	.7905	.7822	.7738	.7654	.7569	.7484	.7398	.7312	.7225
730	.8038	.7955	.7872	.7788	.7703	.7618	.7533	.7447	.7360	.7273
732	.8089	.8005	.7921	.7837	.7752	.7667	.7582	.7496	.7409	.7321
734	.8139	.8055	.7971	.7887	.7802	.7717	.7631	.7545	.7458	.7370
736	.8189	.8105	.8021	.7936	.7851	.7766	.7680	.7593	.7506	.7418
738	.8239	.8155	.8071	.7986	.7901	.7815	.7729	.7642	.7555	.7467
740	.8288	.8204	.8120	.8035	.7950	.7864	.7778	.7691	.7603	.7515
742	.8338	.8254	.8170	.8085	.7999	.7913	.7827	.7740	.7652	.7564
744	.8388	.8304	.8219	.8134	.8048	.7962	.7875	.7788	.7700	.7612
746	.8439	.8354	.8269	.8184	.8098	.8011	.7924	.7837	.7749	.7661
748	.8489	.8404	.8319	.8233	.8147	.8060	.7973	.7886	.7798	.7709
750	.8539	.8454	.8368	.8282	.8196	.8109	.8022	.7934	.7846	.7757
752	.8589	.8504	.8418	.8332	.8246	.8159	.8072	.7984	.7895	.7806
754	.8639	.8554	.8468	.8382	.8295	.8208	.8120	.8032	.7944	.7854
756	.8689	.8603	.8517	.8431	.8344	.8257	.8169	.8081	.7992	.7902
758	.8739	.8653	.8567	.8481	.8394	.8306	.8218	.8130	.8041	.7951
760	.8789	.8703	.8617	.8530	.8443	.8355	.8267	.8178	.8089	.7999
762	.8839	.8753	.8667	.8580	.8492	.8404	.8316	.8227	.8138	.8048
764	.8890	.8803	.8716	.8629	.8541	.8453	.8365	.8276	.8187	.8096
766	.8940	.8853	.8766	.8679	.8591	.8503	.8414	.8325	.8235	.8144
768	.8990	.8903	.8816	.8728	.8640	.8552	.8463	.8374	.8284	.8193
770	.9040	.8953	.8865	.8777	.8689	.8601	.8512	.8422	.8332	.8241

*Density of carbon dioxide (Parr).—Concluded.*

23

20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	mm.
1.6462	1.6370	1.6278	1.6195	1.6112	1.6021	1.5930	1.5837	1.5744	1.5649	1.5554	700
.6510	.6419	.6327	.6243	.6160	.6068	.5977	.5884	.5791	.5696	.5600	702
.6558	.6467	.6376	.6292	.6207	.6116	.6025	.5931	.5838	.5742	.5647	704
.6607	.6516	.6425	.6340	.6254	.6163	.6072	.5979	.5885	.5789	.5693	706
.6655	.6564	.6474	.6388	.6302	.6211	.6119	.6026	.5932	.5836	.5740	708
.6703	.6613	.6522	.6436	.6350	.6258	.6166	.6073	.5978	.5882	.5786	710
.6751	.6662	.6571	.6485	.6397	.6305	.6214	.6120	.6025	.5929	.5832	712
.6799	.6710	.6620	.6533	.6444	.6353	.6261	.6167	.6072	.5976	.5879	714
.6848	.6759	.6670	.6581	.6492	.6400	.6308	.6215	.6119	.6023	.5925	716
.6896	.6807	.6718	.6629	.6540	.6448	.6356	.6262	.6166	.6069	.5972	718
.6944	.6856	.6767	.6678	.6587	.6495	.6403	.6309	.6213	.6116	.6018	720
.6992	.6904	.6815	.6726	.6635	.6543	.6450	.6356	.6260	.6163	.6065	722
.7041	.6953	.6863	.6773	.6682	.6590	.6497	.6403	.6307	.6210	.6111	724
.7089	.7001	.6911	.6821	.6730	.6638	.6544	.6450	.6354	.6256	.6157	726
.7137	.7049	.6959	.6869	.6778	.6685	.6591	.6497	.6401	.6303	.6204	728
.7185	.7097	.7007	.6917	.6825	.6732	.6638	.6544	.6448	.6350	.6251	730
.7233	.7145	.7055	.6964	.6872	.6779	.6685	.6591	.6494	.6396	.6297	732
.7282	.7193	.7103	.7012	.6920	.6827	.6733	.6638	.6541	.6443	.6343	734
.7330	.7241	.7151	.7060	.6968	.6875	.6780	.6685	.6588	.6490	.6390	736
.7378	.7289	.7199	.7107	.7015	.6922	.6827	.6732	.6635	.6537	.6437	738
.7426	.7337	.7247	.7155	.7063	.6969	.6874	.6778	.6681	.6583	.6483	740
.7475	.7385	.7295	.7203	.7111	.7017	.6922	.6826	.6729	.6630	.6530	742
.7523	.7433	.7342	.7250	.7158	.7064	.6969	.6873	.6776	.6677	.6577	744
.7571	.7481	.7390	.7298	.7206	.7112	.7016	.6920	.6822	.6723	.6623	746
.7619	.7529	.7438	.7346	.7253	.7159	.7063	.6967	.6869	.6770	.6670	748
.7667	.7577	.7486	.7394	.7301	.7206	.7110	.7014	.6916	.6817	.6716	750
.7716	.7625	.7534	.7441	.7348	.7254	.7158	.7061	.6963	.6864	.6763	752
.7764	.7673	.7582	.7489	.7396	.7301	.7205	.7108	.7010	.6910	.6809	754
.7812	.7721	.7630	.7537	.7443	.7348	.7252	.7155	.7057	.6957	.6856	756
.7861	.7770	.7678	.7585	.7491	.7396	.7300	.7202	.7104	.7004	.6903	758
.7909	.7818	.7725	.7632	.7538	.7443	.7347	.7249	.7150	.7050	.6949	760
.7957	.7866	.7773	.7680	.7586	.7490	.7394	.7296	.7197	.7097	.6996	762
.8005	.7914	.7821	.7728	.7633	.7538	.7441	.7343	.7244	.7144	.7042	764
.8053	.7962	.7869	.7776	.7681	.7585	.7488	.7390	.7291	.7191	.7089	766
.8102	.8010	.7917	.7823	.7728	.7633	.7535	.7437	.7338	.7237	.7135	768
.8150	.8058	.7965	.7871	.7776	.7680	.7582	.7484	.7385	.7284	.7182	770

24 *Correction factors for the gasometric determination of carbon dioxide.<sup>1</sup>*

(Based on sample weighing 1.7000 grams.)

(Multiply the number of ml of gas evolved from 1.7000 grams of sample by the factor that corresponds with existing atmospheric conditions and divide by ten to obtain percentage of carbon dioxide by weight in sample.)

°C	15.0°	15.5°	16.0°	16.5°	17.0°	17.5°	18.0°	18.5°	
<i>mm.</i>									<i>inches</i>
700	0.99194	0.99006	0.98818	0.98573	0.98329	0.98082	0.97835	0.97585	27.56
702	0.99494	0.99300	0.99106	0.98862	0.98618	0.98368	0.98118	0.97868	27.64
704	0.99794	0.99544	0.99394	0.99147	0.98900	0.98653	0.98406	0.98156	27.72
706	1.00094	0.99886	0.99682	0.99435	0.99188	0.98941	0.98694	0.98440	27.80
708	1.00394	1.00183	0.99971	0.99723	0.99476	0.99226	0.98976	0.98726	27.87
710	1.00694	1.00477	1.00259	1.00012	0.99765	0.99512	0.99259	0.99009	27.95
712	1.00994	1.00767	1.00541	1.00294	1.00047	0.99795	0.99541	0.99291	28.03
714	1.01294	1.01061	1.00829	1.00582	1.00335	1.00080	0.99824	0.99576	28.11
716	1.01594	1.01356	1.01118	1.00871	1.00624	1.00368	1.00112	0.99861	28.19
718	1.01894	1.01650	1.01406	1.01156	1.00906	1.00653	1.00400	1.00150	28.27
720	1.02194	1.01949	1.01694	1.01444	1.01194	1.00941	1.00688	1.00435	28.35
722	1.02482	1.02232	1.01982	1.01732	1.01482	1.01229	1.00976	1.00720	28.43
724	1.02771	1.02521	1.02271	1.02021	1.01771	1.01518	1.01265	1.01009	28.50
726	1.03059	1.02809	1.02559	1.02306	1.02053	1.01800	1.01547	1.01291	28.58
728	1.03347	1.03097	1.02847	1.02594	1.02341	1.02088	1.01835	1.01580	28.66
730	1.03635	1.03385	1.03135	1.02882	1.02629	1.02374	1.02118	1.01862	28.74
732	1.03924	1.03674	1.03424	1.03171	1.02918	1.02662	1.02406	1.02147	28.82
734	1.04218	1.03915	1.03712	1.03459	1.03206	1.02950	1.02694	1.02435	28.90
736	1.04506	1.04253	1.04000	1.03744	1.03488	1.03232	1.02976	1.02718	28.98
738	1.04794	1.04541	1.04288	1.04037	1.03776	1.03521	1.03265	1.03006	29.06
740	1.05082	1.04829	1.04576	1.04321	1.04065	1.03806	1.03547	1.03288	29.13
742	1.05371	1.05118	1.04865	1.04609	1.04353	1.04094	1.03835	1.03577	29.21
744	1.05659	1.05403	1.05147	1.04991	1.04635	1.04377	1.04118	1.03859	29.29
746	1.05947	1.05691	1.05435	1.05180	1.04924	1.04665	1.04406	1.04147	29.37
748	1.06235	1.05929	1.05724	1.05418	1.05212	1.04953	1.04694	1.04433	29.45
750	1.06524	1.06218	1.06012	1.05748	1.05494	1.05235	1.04976	1.04715	29.53
752	1.06818	1.06512	1.06306	1.06047	1.05788	1.05527	1.05265	1.05003	29.61
754	1.07106	1.06847	1.06588	1.06330	1.06071	1.05812	1.05553	1.05289	29.69
756	1.07394	1.07135	1.06876	1.06618	1.06359	1.06197	1.05935	1.05671	29.76
758	1.07682	1.07423	1.07165	1.06906	1.06647	1.06386	1.06124	1.05859	29.84
760	1.07971	1.07712	1.07453	1.07191	1.06929	1.06668	1.06406	1.06141	29.92
762	1.08259	1.08000	1.07741	1.07480	1.07218	1.06956	1.06694	1.06430	30.00
764	1.08547	1.08288	1.08029	1.07768	1.07506	1.07244	1.06982	1.06715	30.08
766	1.08841	1.08580	1.08318	1.08056	1.07794	1.07530	1.07265	1.06997	30.16
768	1.09129	1.08868	1.08606	1.08344	1.08082	1.07818	1.07553	1.07285	30.24
770	1.09418	1.09156	1.08894	1.08630	1.08365	1.08100	1.07835	1.07567	30.31
°F	59.0°	59.9°	60.8°	61.7°	62.6°	63.5°	64.4°	65.3°	

<sup>1</sup> Calculated from 1.976 = weight of 1 liter CO<sub>2</sub> at 0°C, 760 mm pressure and 41° latitude. Formula given by S. W. Parr, *J. Am. Chem. Soc.*, 31, 237 (1909).

Correction factors for the gasometric determination of carbon dioxide.—Continued. 24

°C	19.0°	19.5°	20.0°	20.5°	21.0°	21.5°	22.0°	22.5°	
<i>mm.</i>									<i>inches</i>
700	0.97335	0.97085	0.96835	0.96564	0.96294	0.96023	0.95753	0.95509	27.56
702	0.97618	0.97368	0.97118	0.96850	0.96582	0.96311	0.96041	0.95794	27.64
704	0.97906	0.97653	0.97400	0.97132	0.96865	0.96597	0.96329	0.96082	27.72
706	0.98188	0.97938	0.97688	0.97420	0.97153	0.96888	0.96624	0.96371	27.80
708	0.98476	0.98224	0.97971	0.97703	0.97435	0.97173	0.96912	0.96656	27.87
710	0.98759	0.98506	0.98253	0.97988	0.97724	0.97459	0.97195	0.96938	27.95
712	0.99041	0.98788	0.98535	0.98273	0.98012	0.97747	0.97483	0.97227	28.03
714	0.99329	0.99073	0.98818	0.98556	0.98294	0.98032	0.97771	0.97512	28.11
716	0.99612	0.99358	0.99106	0.98844	0.98582	0.98323	0.98065	0.97800	28.19
718	0.99900	0.99644	0.99388	0.99126	0.98865	0.98606	0.98348	0.98083	28.27
720	1.00182	0.99925	0.99671	0.99412	0.99153	0.98894	0.98636	0.98371	28.35
722	1.00465	1.00209	0.99953	0.99694	0.99435	0.99176	0.98918	0.98653	28.43
724	1.00753	1.00497	1.00241	0.99982	0.99724	0.99462	0.99200	0.98932	28.50
726	1.01035	1.00779	1.00524	1.00265	1.00006	0.99746	0.99483	0.99215	28.58
728	1.01324	1.01065	1.00806	1.00547	1.00288	1.00027	0.99765	0.99497	28.66
730	1.01606	1.01347	1.01088	1.00829	1.00571	1.00306	1.00041	0.99781	28.74
732	1.01888	1.01629	1.01371	1.01112	1.00853	1.00588	1.00324	1.00056	28.82
734	1.02176	1.01919	1.01659	1.01497	1.01135	1.00870	1.00606	1.00338	28.90
736	1.02459	1.02200	1.01941	1.01679	1.01418	1.01153	1.00888	1.00620	28.98
738	1.02747	1.02486	1.02224	1.01962	1.01700	1.01435	1.01171	1.00900	29.06
740	1.03029	1.02768	1.02506	1.02244	1.01982	1.01717	1.01453	1.01182	29.13
742	1.03318	1.03056	1.02794	1.02529	1.02265	1.02000	1.01735	1.01464	29.21
744	1.03600	1.03338	1.03076	1.02811	1.02547	1.02279	1.02212	1.01752	29.29
746	1.03888	1.03624	1.03359	1.03094	1.02829	1.02561	1.02294	1.02024	29.37
748	1.04171	1.03906	1.03641	1.03376	1.03112	1.02844	1.02576	1.02306	29.45
750	1.04453	1.04189	1.03924	1.03659	1.03394	1.03126	1.02859	1.02589	29.53
752	1.04741	1.04477	1.04212	1.03944	1.03676	1.03408	1.03141	1.02868	29.61
754	1.05024	1.04759	1.04494	1.04226	1.03959	1.03691	1.03424	1.03150	29.69
756	1.05306	1.05041	1.04776	1.04508	1.04241	1.03973	1.03706	1.03433	29.76
758	1.05594	1.05330	1.05065	1.04797	1.04529	1.04259	1.03988	1.03715	29.84
760	1.05876	1.05612	1.05347	1.05079	1.04812	1.04539	1.04265	1.03992	29.92
762	1.06165	1.05897	1.05629	1.05361	1.05094	1.04821	1.04547	1.04274	30.00
764	1.06447	1.06179	1.05912	1.05644	1.05376	1.05103	1.04829	1.04556	30.08
766	1.06729	1.06462	1.06194	1.05926	1.05659	1.05386	1.05112	1.04839	30.16
768	1.07018	1.06750	1.06482	1.06212	1.05941	1.05668	1.05394	1.05118	30.24
770	1.07300	1.07032	1.06765	1.06494	1.06224	1.05950	0.05676	1.05400	30.31
°F.	66.2°	67.1°	68.0°	68.9°	69.8°	70.7°	71.6°	72.5°	

24 *Correction factors for the gasometric determination of carbon dioxide.*—Continued.

°C	23.0°	23.5°	24.0°	24.5°	25.0°	25.5°	26.0°	26.5°	
<i>mm.</i>									<i>inches</i>
700	0.95265	0.95020	0.94776	0.94508	0.94241	0.93973	0.93706	0.93432	27.56
702	0.95547	0.95303	0.95059	0.94788	0.94518	0.94250	0.93982	0.93708	27.64
704	0.95835	0.95585	0.95335	0.95067	0.94800	0.94532	0.94265	0.93988	27.72
706	0.96118	0.95865	0.95612	0.95344	0.95076	0.94808	0.94541	0.94267	27.80
708	0.96400	0.96147	0.95894	0.95626	0.95359	0.95088	0.94818	0.94544	27.87
710	0.96682	0.96429	0.96176	0.95905	0.95635	0.95364	0.95094	0.94820	27.95
712	0.96971	0.96712	0.96453	0.96182	0.95912	0.95644	0.95376	0.95100	28.03
714	0.97253	0.96991	0.96729	0.96461	0.96194	0.95923	0.95653	0.95376	28.11
716	0.97535	0.97273	0.97012	0.96741	0.96471	0.96200	0.95929	0.95655	28.19
718	0.97818	0.97556	0.97294	0.97023	0.96753	0.96482	0.96212	0.95935	28.27
720	0.98106	0.97838	0.97571	0.97300	0.97029	0.96758	0.96488	0.96213	28.35
722	0.98388	0.98120	0.97853	0.97582	0.97312	0.97038	0.96765	0.96488	28.43
724	0.98665	0.98397	0.98129	0.97858	0.97588	0.97314	0.97041	0.96764	28.50
726	0.98947	0.98679	0.98412	0.98141	0.97871	0.97594	0.97318	0.97041	28.58
728	0.99229	0.98961	0.98694	0.98420	0.98147	0.97870	0.97594	0.97319	28.66
730	0.99512	0.99241	0.98971	0.98697	0.98424	0.98147	0.97871	0.97594	28.74
732	0.99788	0.99517	0.99247	0.98973	0.98700	0.98423	0.98147	0.97871	28.82
734	1.00071	0.99799	0.99529	0.99255	0.98982	0.98705	0.98429	0.98155	28.90
736	1.00353	1.00083	0.99812	0.99538	0.99265	0.98985	0.98706	0.98426	28.98
738	1.00629	1.00359	1.00088	0.99815	0.99541	0.99261	0.98982	0.98703	29.06
740	1.00912	1.00643	1.00371	1.00095	0.99818	0.99538	0.99259	0.98976	29.13
742	1.01194	1.00923	1.00653	1.00377	1.00100	0.99820	0.99541	0.99258	29.21
744	1.01471	1.01200	1.00929	1.00653	1.00376	1.00097	0.99818	0.99535	29.29
746	1.01753	1.01482	1.01212	1.00936	1.00659	1.00376	1.00094	0.99809	29.37
748	1.02035	1.01762	1.01488	1.01212	1.00935	1.00653	1.00371	1.00088	29.45
750	1.02318	1.02045	1.01771	1.01492	1.01212	1.00936	1.00659	1.00370	29.53
752	1.02594	1.02321	1.02047	1.01771	1.01494	1.01211	1.00929	1.00644	29.61
754	1.02876	1.02603	1.02329	1.02050	1.01771	1.01483	1.01206	1.00921	29.69
756	1.03159	1.02883	1.02606	1.02326	1.02047	1.01764	1.01482	1.01197	29.76
758	1.03441	1.03165	1.02888	1.02608	1.02329	1.02047	1.01765	1.01477	29.84
760	1.03718	1.03442	1.03165	1.02886	1.02606	1.02323	1.02041	1.01753	29.92
762	1.04000	1.03724	1.03447	1.03164	1.02882	1.02600	1.02318	1.02030	30.00
764	1.04282	1.04003	1.03723	1.03444	1.03165	1.02880	1.02594	1.02306	30.08
766	1.04565	1.04285	1.04005	1.03723	1.03441	1.03156	1.02871	1.02583	30.16
768	1.04841	1.04562	1.04282	1.04003	1.03724	1.03435	1.03147	1.02859	30.24
770	1.05123	1.04844	1.04564	1.04282	1.04000	1.03712	1.03424	1.03136	30.31
°F.	73.4°	74.3°	75.2°	76.1°	77.0°	77.9°	78.8°	79.7°	

## Correction factors for the gasometric determination of carbon dioxide.—Continued. 24

°C	27.0°	27.5°	28.0°	28.5°	29.0°	29.5°	30.0°	30.5°	
<i>mm.</i>									<i>inches</i>
700	0.93159	0.92885	0.92612	0.92332	0.92053	0.91773	0.91494	0.91203	27.56
702	0.93435	0.92161	0.92888	0.92608	0.92329	0.92047	0.91765	0.91476	27.64
704	0.93712	0.93438	0.93165	0.92882	0.92600	0.92320	0.92041	0.91750	27.72
706	0.93994	0.93717	0.93441	0.93158	0.92876	0.92594	0.92312	0.92024	27.80
708	0.94271	0.93994	0.93718	0.93435	0.93153	0.92870	0.92588	0.92297	27.87
710	0.94547	0.94267	0.93988	0.93706	0.93424	0.93141	0.92859	0.92567	27.95
712	0.94824	0.94544	0.94265	0.93982	0.93700	0.93414	0.93129	0.92841	28.03
714	0.95100	0.94820	0.94541	0.94258	0.93976	0.93691	0.93406	0.93115	28.11
716	0.95382	0.95100	0.94818	0.94535	0.94253	0.93964	0.93676	0.93388	28.19
718	0.95659	0.95376	0.95094	0.94809	0.94524	0.94238	0.93953	0.93662	28.27
720	0.95939	0.95655	0.95371	0.95085	0.94800	0.94512	0.94224	0.93932	28.35
722	0.96212	0.95929	0.95647	0.95361	0.95076	0.94788	0.94500	0.94209	28.43
724	0.96488	0.96206	0.95924	0.95638	0.95353	0.95062	0.94771	0.94479	28.50
726	0.96765	0.96482	0.96200	0.95912	0.95624	0.95332	0.95041	0.94750	28.58
728	0.97041	0.96758	0.96476	0.96188	0.95900	0.95609	0.95318	0.95026	28.66
730	0.97318	0.97036	0.96753	0.96464	0.96176	0.95885	0.95594	0.95300	28.74
732	0.97594	0.97309	0.97024	0.96735	0.96447	0.96156	0.95865	0.95578	28.82
734	0.97871	0.97585	0.97300	0.97012	0.96724	0.96429	0.96135	0.95844	28.90
736	0.98147	0.97861	0.97576	0.97288	0.97000	0.96706	0.96412	0.96118	28.98
738	0.98424	0.98138	0.97853	0.97564	0.97276	0.96982	0.96688	0.96394	29.06
740	0.98694	0.98409	0.98124	0.97835	0.97547	0.97253	0.96959	0.96665	29.13
742	0.98976	0.98691	0.98406	0.98115	0.97824	0.97529	0.97235	0.96941	29.21
744	0.99253	0.98967	0.98682	0.98391	0.98100	0.97806	0.97512	0.97215	29.29
746	0.99529	0.99241	0.98953	0.98662	0.98371	0.98076	0.97782	0.97485	29.37
748	0.99806	0.99517	0.99229	0.98938	0.98647	0.98353	0.98059	0.97762	29.45
750	1.00082	0.99796	0.99506	0.99215	0.98924	0.98626	0.98329	0.98032	29.53
752	1.00359	1.00071	0.99782	0.99491	0.99200	0.98903	0.98606	0.98306	29.61
754	1.00635	1.00342	1.00050	0.99758	0.99471	0.99173	0.98876	0.98579	29.69
756	1.00912	1.00624	1.00335	1.00041	0.99747	0.99450	0.99153	0.98853	29.76
758	1.01188	1.00900	1.00612	1.00318	1.00024	0.99724	0.99429	0.99129	29.84
760	1.01465	1.01174	1.00882	1.00588	1.00294	0.99995	0.99700	0.99400	29.92
762	1.01741	1.01450	1.01159	1.00865	1.00571	1.00274	0.99976	0.99673	30.00
764	1.02018	1.01727	1.01435	1.01141	1.00847	1.00547	1.00247	0.99948	30.08
766	1.02294	1.02003	1.01712	1.01418	1.01124	1.00824	1.00524	1.00221	30.16
768	1.02571	1.02280	1.01988	1.01694	1.01394	1.01094	1.00794	1.00491	30.24
770	1.02847	1.02556	1.02265	1.01968	1.01671	1.01371	1.01071	1.00768	30.31
°F	80.6°	81.5°	82.4°	83.3°	84.2°	85.1°	86.0°	86.9°	

24 Correction factors for the gasometric determination of carbon dioxide.—Concluded.

°C	31.0°	31.5°	32.0°	32.5°	33.0°	33.5°	34.0°	34.5°	35.0°	
<i>mm.</i>										<i>inches</i>
700	0.90912	0.90620	0.90329	0.90082	0.89735	0.89432	0.89129	0.88821	0.88512	27.56
702	0.91188	0.90894	0.90600	0.90303	0.90006	0.89703	0.89400	0.89091	0.88782	27.64
704	0.91459	0.91165	0.90871	0.90576	0.90282	0.89976	0.89671	0.89362	0.89053	27.72
706	0.91735	0.91441	0.91147	0.90847	0.90547	0.90241	0.89935	0.89627	0.89318	27.80
708	0.92006	0.91712	0.91418	0.91118	0.90818	0.90512	0.90206	0.89897	0.89588	27.87
710	0.92276	0.91982	0.91688	0.91388	0.91088	0.90782	0.90476	0.90168	0.89859	27.95
712	0.92553	0.92256	0.91959	0.91659	0.91359	0.91053	0.90747	0.90438	0.90129	28.03
714	0.92824	0.92529	0.92235	0.91932	0.91629	0.91323	0.91018	0.90706	0.90394	28.11
716	0.93100	0.92803	0.92506	0.92203	0.91900	0.91594	0.91288	0.90976	0.90665	28.19
718	0.93371	0.93078	0.92776	0.92474	0.92171	0.91865	0.91559	0.91247	0.90935	28.27
720	0.93641	0.93344	0.93047	0.92744	0.92441	0.92135	0.91829	0.91517	0.91206	28.35
722	0.93918	0.93618	0.93318	0.93015	0.92712	0.92412	0.92100	0.91785	0.91471	28.43
724	0.94188	0.93887	0.93606	0.93294	0.92982	0.92676	0.92371	0.92056	0.91741	28.50
726	0.94459	0.94159	0.93859	0.93556	0.93253	0.92944	0.92635	0.92323	0.92012	28.58
728	0.94735	0.94435	0.94135	0.93830	0.93544	0.93215	0.92906	0.92591	0.92276	28.66
730	0.95006	0.94706	0.94406	0.94103	0.93800	0.93488	0.93176	0.92861	0.92547	28.74
732	0.95282	0.94979	0.94676	0.94373	0.94071	0.93759	0.93447	0.93132	0.92818	28.82
734	0.95553	0.95250	0.94947	0.94644	0.94341	0.94034	0.93718	0.93403	0.93088	28.90
736	0.95824	0.95521	0.95218	0.94915	0.94612	0.94300	0.93988	0.93670	0.93353	28.98
738	0.96100	0.95797	0.95494	0.95188	0.94882	0.94570	0.94259	0.93941	0.93624	29.06
740	0.96371	0.96068	0.95765	0.95459	0.95153	0.94841	0.94529	0.94211	0.93894	29.13
742	0.96647	0.96341	0.96035	0.95730	0.95424	0.95112	0.94800	0.94482	0.94165	29.21
744	0.96918	0.96615	0.96312	0.96003	0.95694	0.95382	0.95071	0.94750	0.94429	29.29
746	0.97188	0.96885	0.96582	0.96273	0.95965	0.95653	0.95341	0.95020	0.94700	29.37
748	0.97465	0.97159	0.96853	0.96544	0.96235	0.95925	0.95606	0.95288	0.94971	29.45
750	0.97735	0.97429	0.97124	0.96815	0.96506	0.96191	0.95876	0.95558	0.95241	29.53
752	0.98006	0.97703	0.97400	0.97088	0.96776	0.96461	0.96147	0.95826	0.95506	29.61
754	0.98282	0.97976	0.97671	0.97359	0.97047	0.96732	0.96418	0.96097	0.95776	29.69
756	0.98553	0.98247	0.97941	0.97629	0.97318	0.97003	0.96688	0.96367	0.96047	29.76
758	0.98829	0.98521	0.98212	0.97900	0.97588	0.97273	0.96959	0.96638	0.96318	29.84
760	0.99100	0.98794	0.98488	0.98176	0.97865	0.97547	0.97229	0.96908	0.96588	29.92
762	0.99371	0.99065	0.98759	0.98443	0.98135	0.97817	0.97500	0.97176	0.96853	30.00
764	0.99647	0.99338	0.99029	0.98717	0.98406	0.98088	0.97771	0.97447	0.97124	30.08
766	0.99918	0.99609	0.99300	0.98988	0.98676	0.98356	0.98035	0.97714	0.97394	30.16
768	1.00188	0.99880	0.99571	0.99259	0.98947	0.98629	0.98312	0.97986	0.97659	30.24
770	1.00465	1.00156	0.99847	0.99532	0.99218	0.98897	0.98576	0.98252	0.97929	30.31
°F.	87.8°	88.7°	89.6°	90.5°	91.4°	92.3°	93.2°	94.1°	95.0°	



## Progressive accumulation of radium emanation\*

25

$$I_t = I_0(1 - e^{-\lambda t}); \lambda = 0.1801 \text{ days}^{-1}; I_0 = 1$$

HOURS	0 DAYS	$\Delta$	1 DAY	$\Delta$	2 DAYS	$\Delta$	3 DAYS	$\Delta$	4 DAYS	$\Delta$	5 DAYS	$\Delta$	6 DAYS	$\Delta$	7 DAYS	$\Delta$
0	.0000	75	.1648	63	.3025	52	.4175	44	.5136	36	.5937	31	.6607	25	.7166	22
1	.0075	74	.1711	62	.3077	52	.4219	43	.5172	36	.5968	30	.6632	26	.7188	20
2	.0149	74	.1773	61	.3129	52	.4262	43	.5208	36	.5998	30	.6658	24	.7208	21
3	.0223	73	.1834	62	.3181	51	.4305	42	.5244	35	.6028	29	.6682	25	.7229	21
4	.0296	72	.1896	60	.3232	50	.4347	42	.5279	35	.6057	30	.6707	25	.7250	21
5	.0368	72	.1956	60	.3282	50	.4390	42	.5314	36	.6087	29	.6732	24	.7271	20
6	.0440	72	.2016	60	.3332	50	.4432	41	.5350	34	.6116	29	.6756	25	.7291	21
7	.0512	71	.2076	59	.3382	50	.4473	41	.5384	35	.6145	29	.6781	24	.7311	20
8	.0583	70	.2135	59	.3432	49	.4514	42	.5419	34	.6174	29	.6805	24	.7332	19
9	.0653	70	.2194	58	.3481	49	.4556	40	.5453	34	.6203	28	.6829	23	.7351	20
10	.0723	69	.2252	58	.3530	48	.4596	41	.5487	34	.6231	28	.6852	24	.7371	20
11	.0792	69	.2310	58	.3578	48	.4637	40	.5521	33	.6259	28	.6876	23	.7391	20
12	.0861	69	.2368	57	.3626	48	.4677	40	.5554	34	.6287	28	.6899	23	.7410	20
13	.0930	68	.2425	57	.3674	47	.4717	39	.5588	33	.6315	27	.6922	23	.7430	19
14	.0998	67	.2482	56	.3721	47	.4756	39	.5621	32	.6342	28	.6945	23	.7449	19
15	.1065	67	.2538	56	.3768	46	.4795	39	.5653	33	.6370	27	.6968	23	.7468	19
16	.1132	66	.2594	55	.3814	47	.4834	39	.5686	32	.6397	27	.6991	23	.7487	19
17	.1198	66	.2649	55	.3861	46	.4873	38	.5718	32	.6424	27	.7014	22	.7506	18
18	.1264	65	.2704	54	.3907	45	.4911	38	.5750	32	.6451	26	.7036	22	.7524	19
19	.1329	65	.2758	55	.3952	46	.4949	38	.5782	31	.6477	27	.7058	22	.7543	18
20	.1394	64	.2813	53	.3998	44	.4987	37	.5813	32	.6504	26	.7080	22	.7561	19
21	.1458	64	.2866	54	.4042	45	.5024	38	.5845	31	.6530	26	.7102	22	.7580	18
22	.1522	64	.2920	53	.4087	44	.5062	37	.5876	31	.6556	25	.7124	21	.7598	18
23	.1586	62	.2973	52	.4131	44	.5099	37	.5907	30	.6581	26	.7145	21	.7616	18

HOURS	8 DAYS	$\Delta$	9 DAYS	$\Delta$	10 DAYS	$\Delta$	11 DAYS	$\Delta$	12 DAYS	$\Delta$	13 DAYS	$\Delta$	14 DAYS	$\Delta$	15 DAYS	$\Delta$
0	.7634	17	.8024	14	.8349	13	.8622	10	.8849	8	.9038	8	.9197	6	.9329	5
1	.7651	18	.8038	15	.8362	12	.8632	10	.8857	9	.9046	7	.9203	6	.9334	5
2	.7669	17	.8053	15	.8374	12	.8642	10	.8866	8	.9053	7	.9209	6	.9339	5
3	.7686	18	.8068	14	.8386	12	.8652	10	.8874	9	.9060	7	.9215	6	.9344	5
4	.7704	17	.8082	14	.8398	12	.8662	10	.8883	8	.9067	7	.9221	6	.9349	5
5	.7721	17	.8096	15	.8410	12	.8672	10	.8891	8	.9074	7	.9227	5	.9354	5
6	.7738	17	.8111	14	.8422	12	.8682	10	.8899	9	.9081	7	.9232	6	.9359	5
7	.7755	16	.8125	15	.8434	12	.8692	10	.8908	8	.9088	7	.9238	6	.9364	4
8	.7771	17	.8139	14	.8446	11	.8702	10	.8916	8	.9095	6	.9244	6	.9368	5
9	.7788	17	.8153	13	.8457	12	.8712	9	.8924	8	.9101	7	.9250	5	.9373	5
10	.7805	16	.8166	14	.8469	11	.8721	10	.8932	8	.9108	7	.9255	6	.9378	4
11	.7821	16	.8180	14	.8480	12	.8731	9	.8940	8	.9115	6	.9261	5	.9382	5
12	.7837	17	.8194	13	.8492	11	.8740	10	.8948	8	.9121	7	.9266	6	.9387	5
13	.7854	16	.8207	14	.8503	11	.8750	9	.8956	8	.9128	6	.9272	5	.9392	4
14	.7870	16	.8221	13	.8514	11	.8759	9	.8964	7	.9134	7	.9277	5	.9396	4
15	.7886	15	.8234	13	.8525	11	.8768	9	.8971	8	.9141	6	.9282	6	.9401	4
16	.7901	16	.8247	13	.8536	11	.8777	10	.8979	8	.9147	7	.9288	5	.9405	5
17	.7917	15	.8260	13	.8547	11	.8787	9	.8987	7	.9154	6	.9293	5	.9410	4
18	.7932	16	.8273	13	.8558	11	.8796	9	.8994	8	.9160	6	.9298	6	.9414	4
19	.7948	15	.8286	13	.8569	11	.8805	9	.9002	7	.9166	7	.9304	5	.9418	5
20	.7963	16	.8299	13	.8580	10	.8814	8	.9009	8	.9173	6	.9309	5	.9423	4
21	.7979	15	.8312	12	.8590	11	.8822	9	.9017	7	.9179	6	.9314	5	.9427	5
22	.7994	15	.8324	13	.8601	10	.8831	9	.9024	7	.9185	6	.9319	5	.9432	4
23	.8009	15	.8337	12	.8611	11	.8840	9	.9031	7	.9191	6	.9324	5	.9436	4

25

*Progressive accumulation of radium emanation—Concluded.*

HOURS	16 DAYS	$\Delta$	17 DAYS	$\Delta$	18 DAYS	$\Delta$	19 DAYS	$\Delta$	20 DAYS	$\Delta$	21 DAYS	$\Delta$	22 DAYS	$\Delta$	23 DAYS	$\Delta$
0	.9440	9	.9533	7	.9610	6	.9674	5	.9728	4	.9773	3	.9810	3	.9842	2
2	.9449	8	.9540	6	.9616	5	.9679	5	.9732	4	.9776	4	.9813	3	.9844	2
4	.9457	8	.9546	7	.9621	6	.9684	5	.9736	4	.9780	3	.9816	3	.9846	3
6	.9465	8	.9553	7	.9627	6	.9689	4	.9740	4	.9783	3	.9819	3	.9849	2
8	.9473	8	.9560	6	.9633	5	.9693	5	.9744	4	.9786	3	.9822	2	.9851	2
10	.9481	8	.9566	7	.9638	5	.9698	4	.9748	4	.9789	3	.9824	3	.9853	2
12	.9489	7	.9573	6	.9643	6	.9702	5	.9752	3	.9792	4	.9827	2	.9855	2
14	.9496	8	.9579	7	.9649	5	.9707	4	.9755	4	.9796	3	.9829	3	.9857	2
16	.9504	7	.9586	6	.9654	5	.9711	4	.9759	3	.9799	3	.9832	2	.9859	2
18	.9511	7	.9592	6	.9659	5	.9715	5	.9762	4	.9802	3	.9834	3	.9861	2
20	.9518	8	.9598	6	.9664	5	.9720	4	.9766	3	.9805	3	.9837	2	.9863	2
22	.9526	7	.9604	6	.9669	5	.9724	4	.9769	4	.9808	2	.9839	3	.9865	2

HOURS	24 DAYS	$\Delta$	25 DAYS	$\Delta$	26 DAYS	$\Delta$	27 DAYS	$\Delta$	28 DAYS	$\Delta$	29 DAYS	$\Delta$	30 DAYS	$\Delta$	31 DAYS	$\Delta$
0	.9867	4	.9889	4	.9908	2	.9923	2	.9936	1	.9947	1	.9955	2	.9963	1
4	.9871	4	.9893	3	.9910	3	.9925	2	.9937	2	.9948	2	.9957	1	.9964	1
8	.9875	4	.9896	3	.9913	3	.9927	2	.9939	2	.9950	1	.9958	1	.9965	1
12	.9879	3	.9899	3	.9916	2	.9929	3	.9941	2	.9951	2	.9959	1	.9966	1
16	.9882	4	.9902	3	.9918	2	.9932	2	.9943	2	.9953	1	.9960	2	.9967	1
20	.9886	3	.9905	3	.9920	3	.9934	2	.9945	2	.9954	1	.9962	1	.9968	1

\* Univ. of Missouri Bull., 24, No. 26, 85 (1923).

## APPENDIX

### DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

#### DEFINITIONS

##### ACID-FORMING AND NON-ACID-FORMING FERTILIZERS

An *acid-forming fertilizer* is one that is capable of increasing the residual acidity of soil (adopted 1936).

A *non-acid-forming fertilizer* is one that is not capable of increasing the residual acidity of the soil (adopted 1936).

##### ACIDULATED FISH TANKAGE, ACIDULATED FISH SCRAP

*Acidulated fish tankage, acidulated fish scrap*, is the rendered product derived from fish and treated with sulfuric acid (adopted 1930).

##### ACTIVATED SEWAGE PRODUCTS

*Activated sewage products* are those made from sewage freed from grit and coarse solids and aerated after being inoculated with microorganisms. The resulting flocculated organic matter is withdrawn from the tanks, filtered with or without the aid of coagulants, dried, ground, and screened (adopted 1930).

##### AGRICULTURAL LIMING MATERIAL

*Agricultural liming material* is material whose calcium and magnesium content is capable of neutralizing soil acidity (adopted 1935).

##### AIR-SLAKED LIME

*Air-slaked lime* is a product composed of varying proportions of the oxide, hydroxide, and carbonate of calcium, or of calcium and magnesium, and derived from exposure of quicklime (adopted 1936).

##### AMMONIATED SUPERPHOSPHATE

*Ammoniated superphosphate* is the product obtained when superphosphate is treated with ammonia or with a solution containing free ammonia and other forms of nitrogen dissolved therein (adopted 1939, first action).

##### ANALYSIS

The word *analysis*, as applied to fertilizers, shall designate the percentage composition of the product expressed in those terms that the law requires and permits (adopted 1938).

##### ASHES FROM LEACHED WOOD

*Ashes from leached wood* are unleached ashes resulting from burning wood that has been exposed to or digested in water or other liquid solvent, as in the extraction of dyes, so that a part of the plant food has been dissolved and removed (adopted 1926).

##### AVAILABLE PHOSPHORIC ACID

*Available phosphoric acid* is the sum of the water-soluble and the citrate-soluble phosphoric acid (adopted 1931).

**"BASIC" LIME PHOSPHATE**

"Basic" lime phosphate (lime-based superphosphate) is a superphosphate to which lining materials have been added in a quantity at least six per cent (6%) calcium carbonate equivalents in excess of the quantity required to convert all water-soluble phosphate to the citrate-soluble form (adopted 1934).

**BASIC PHOSPHATE SLAG**

*Basic phosphate slag* is a by-product in the manufacture of steel from phosphatic iron ores. The product shall be finely ground and shall contain no admixture of materials other than what results in the original process of manufacture. It shall contain not less than twelve per cent (12%) of total phosphoric acid ( $P_2O_5$ ), not less than eighty per cent (80%) of which shall be soluble in two per cent (2%) citric acid solution according to the Wagner method of analysis, II, 62 and 64 or 65. Any phosphate slag not conforming to this definition shall be designated *low grade* (adopted 1925).

**BAT GUANO**

*Bat guano* is partially decomposed bat manure (adopted 1938).

**BAT MANURE**

*Bat manure* is the dry excrement from bats (adopted 1938).

**BRAND AND BRAND NAME**

A *brand* is a term, design, or trademark used in connection with one or several grades of fertilizers (adopted 1926).

A *brand name* is a specific designation applied to an individual fertilizer (adopted 1926).

**CALCIUM NITRATE**

*Calcium nitrate* (nitrate of lime) is a commercial product consisting chiefly of calcium nitrate, and it shall contain not less than fifteen per cent (15%) of nitrogen (adopted 1939, first action).

**CITRATE-SOLUBLE ("REVERTED") PHOSPHORIC ACID**

*Citrate-soluble* ("reverted") phosphoric acid is that part of the total phosphoric acid in a fertilizer that is insoluble in water but soluble in a solution of citrate of ammonia according to the method adopted by the A.O.A.C. (adopted 1932).

**CRUDE, INERT, OR SLOW-ACTING NITROGENOUS MATERIALS**

*Crude, inert, or slow-acting nitrogenous materials* are unprocessed organic substances relatively high in nitrogen but having a very low value as plant food and showing a low activity by both the alkaline and neutral permanganate methods below 50% and 80%, respectively (adopted 1929).

**CYANAMID**

*Cyanamid* is a commercial product composed chiefly of calcium cyanamide ( $CaCN_2$ ), and it shall contain not less than twenty-one per cent (21%) of nitrogen (adopted 1935).

**DICALCIUM PHOSPHATE**

*Dicalcium phosphate* is a manufactured product consisting chiefly of a dicalcium salt of phosphoric acid (adopted 1931).

**DISSOLVED BONE**

*Dissolved bone* is ground bone or bone meal that has been treated with sulfuric acid (adopted 1926).

**DOLOMITE**

*Dolomite* is a mineral composed chiefly of carbonates of magnesium and calcium in substantially unimolal (1-1.19) proportions (adopted 1938).

**DRIED BLOOD**

*Dried blood* is the collected blood of slaughtered animals, dried and ground and containing not less than twelve per cent (12%) of nitrogen in organic forms (adopted 1928).

**DRIED, PULVERIZED, OR SHREDDED MANURES**

*Dried, pulverized, or shredded manures* are what the name indicates, and not mixtures of manures and other materials (adopted 1925).

**FERTILIZER GRADE**

*Fertilizer grade* shall represent the minimum guaranty of its plant food expressed in terms of *nitrogen (not ammonia)*, *available phosphoric acid*, and *water-soluble potash* (adopted 1928).

**FISH TANKAGE, FISH SCRAP, DRY GROUND FISH, FISH MEAL FERTILIZER GRADE**

*Fish tankage, fish scrap, dry ground fish, fish meal fertilizer grade*, is the dried ground product derived from rendered or unrendered fish (adopted 1929).

**GARBAGE TANKAGE**

*Garbage tankage* is the rendered, dried, and ground product derived from waste household food materials (adopted 1929).

**PULVERIZED LIMESTONE (FINE-GROUND LIMESTONE)**

*Pulverized limestone (fine-ground limestone)* is the product obtained by grinding either calcitic or dolomitic limestone so that all the material will pass a 20-mesh sieve and at least seventy-five per cent (75%) will pass a 100-mesh sieve (adopted 1936).

**GROUND LIMESTONE (COARSE-GROUND LIMESTONE)**

*Ground limestone (coarse-ground limestone)* is the product obtained by grinding either calcitic or dolomitic limestone so that all the material will pass a 10-mesh sieve, and at least fifty per cent (50%) will pass a 100-mesh sieve (adopted 1936).

**GROUND SHELLS**

*Ground shells* is the product obtained by grinding the shells of mollusks so that not less than fifty per cent (50%) shall pass a 100-mesh sieve. The product shall also carry the name of the mollusk from which said product is made (adopted 1936).

**GROUND SHELL MARL**

*Ground shell marl* is the product obtained by grinding natural deposits of shell marl so that at least seventy-five per cent (75%) shall pass a 100-mesh sieve (adopted 1936).

**GROUND RAW BONE**

*Ground raw bone* is dried ground animal bones that have not been previously steamed under pressure (adopted 1929).

## GROUND STEAMED BONE

*Ground steamed bone* is ground animal bones that have been previously steamed under pressure (adopted 1929).

## GYPSUM, LAND PLASTER, OR CRUDE CALCIUM SULFATE

*Gypsum, land plaster, or crude calcium sulfate* are products consisting chiefly of calcium sulfate. They may contain twenty per cent (20%) of combined water. (They do not neutralize acid soils) (adopted 1931).

## HIGH CALCIC PRODUCTS

*High calcic products* are materials of which 90% or more of the total calcium and magnesium content consists of calcium oxide (adopted 1935).

## HIGH MAGNESIC PRODUCTS

*High magnesian products* are materials in which more than 10 per cent of the total calcium and magnesium oxide consists of magnesium oxide (adopted 1935).

## HOOF AND HORN MEAL

*Hoof and horn meal* is processed dried, ground hoofs and horns (adopted 1929).

## HYDRATED OR SLAKED LIME

*Hydrated or slaked lime* is a dry product consisting chiefly of the hydroxide of calcium and oxide-hydroxide of magnesium (adopted 1935).

## KAINIT

*Kainit* is a potash salt containing potassium and sodium chlorides and sometimes sulfate of magnesia with not less than twelve per cent (12%) of potash ( $K_2O$ ) (adopted 1928).

## LEACHED WOOD ASHES

*Leached wood ashes* are ashes from burned unleached wood with part of their plant food removed by artificial means or by exposure to rains, snows, or other solvent (adopted 1928).

## LIME

The word *lime* when applied to liming materials means either calcium oxide or calcium and magnesium oxides (adopted 1934).

## MANGANESE

*Manganese*. The water-soluble (or available) manganese in fertilizers shall be expressed as manganese (Mn) (adopted 1935).

## MANGANESE SULFATE

*Manganese sulfate*. The term manganese sulfate, when applied to an ingredient of a mixed fertilizer, shall designate anhydrous manganous sulfate ( $MnSO_4$ ) (adopted 1935).

## MANURE SALTS

*Manure salts* are potash salts containing high percentages of chloride and from twenty per cent (20%) to thirty per cent (30%) of potash ( $K_2O$ ). The term *double manure salts* should be discontinued (adopted 1925).

## MONOAMMONIUM PHOSPHATE (FERTILIZER GRADE)

*Monoammonium phosphate (fertilizer grade)* is a commercial salt made by combining phosphoric acid with ammonia. It shall contain not less than ten per cent (10%) of nitrogen and not less than forty-six per cent (46%) of available phosphoric acid (adopted 1934).

## MURIATE OF POTASH (COMMERCIAL POTASSIUM CHLORIDE)

*Muriate of potash* (commercial potassium chloride) is a potash salt containing not less than forty-eight per cent (48%) of potash ( $K_2O$ ), chiefly as chlorides (adopted 1929).

## NITRATE OF POTASH (COMMERCIAL POTASSIUM NITRATE)

*Nitrate of potash* (commercial potassium nitrate) is a salt containing not less than twelve per cent (12%) of nitrogen and forty-four per cent (44%) of potash ( $K_2O$ ) (adopted 1927).

## NITRATE OF SODA (COMMERCIAL SODIUM NITRATE)

*Nitrate of soda* (commercial sodium nitrate) is commercial sodium nitrate containing not less than fifteen per cent (15%) of nitrogen, chiefly as sodium nitrate (adopted 1928).

## PEAT

*Peat* is partly decayed vegetable matter of natural occurrence. It is composed chiefly of organic matter that contains some nitrogen of low activity (adopted 1931).

## CHARRED PEAT

*Charred peat* is peat artificially dried at a temperature that causes partial decomposition (adopted 1931).

## PHOSPHATE ROCK

*Phosphate rock* is a natural rock containing one or more calcium phosphate minerals of sufficient purity and quantity to permit its use, either directly or after concentration, in the manufacture of commercial products (adopted 1933).

## PHOSPHORIC ACID

The term *phosphoric acid* designates phosphorus pentoxide ( $P_2O_5$ ) (adopted 1934).

## POTASH

The term *potash* designates potassium oxide ( $K_2O$ ) (adopted 1934).

## PRECIPITATED BONE PHOSPHATE

*Precipitated bone phosphate* is a by-product from the manufacture of glue from bones and is obtained by neutralizing the hydrochloric acid solution of processed bone with calcium hydroxide. The phosphoric acid is chiefly present as dicalcium phosphate (adopted 1933).

## PRECIPITATED PHOSPHATE

*Precipitated phosphate* is a product consisting mainly of dicalcium phosphate obtained by neutralizing with calcium hydroxide the acid solution of either phosphate rock or processed bone (adopted 1933).

## PRIMARY FERTILIZER COMPONENTS

*Primary fertilizer components* are those at present generally recognized by law as necessary to be guaranteed in fertilizers, namely: nitrogen, phosphoric acid ( $P_2O_5$ ), and potash ( $K_2O$ ) (adopted 1938).

## SECONDARY FERTILIZER COMPONENTS

*Secondary fertilizer components* are those other than the "primary fertilizer components" that are essential to the proper growth of plants and that may be needed by some soils. Some of these components are calcium, magnesium, sulfur, manganese, copper, zinc, and boron (adopted 1938).

## PROCESS TANKAGES

*Process tankages* are products made under steam pressure from crude inert nitrogenous materials, with or without the use of acids, for the purpose of increasing the activity of the nitrogen. These products shall be called "Process Tankages" with or without further qualification. The water-insoluble nitrogen in these products shall test at least fifty per cent (50%) active by the alkaline, or eighty per cent (80%) by the neutral permanganate method (adopted 1931).

## PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALI SALTS CONTAINING POTASH

*Products secured by heating calcium phosphate with alkali salts containing potash* are non-acid phosphates with potash. They are *not* potassium phosphate (adopted 1928).

## QUICK LIME, BURNED LIME, CAUSTIC LIME, LUMP LIME, UNSLAKED LIME

*Quick lime, burned lime, caustic lime, lump lime, unslaked lime.* These designations shall apply to calcined materials, the major part of which is calcium oxide, in natural association with a lesser amount of magnesium oxide, and which is capable of slaking with water (adopted 1935).

## SHEEP MANURE—WOOL WASTE

*Sheep manure—wool waste* is the by-product from wool-carding establishments consisting chiefly of sheep manure, seeds, and wool fiber (adopted 1931).

## SOFT PHOSPHATE WITH COLLOIDAL CLAY

*Soft phosphate with colloidal clay* is a very finely divided low-analysis by-product from mining Florida rock phosphate by a hydraulic process in which the colloidal materials settle at points in artificial ponds and basins farthest from the washer, and are later removed after the natural evaporation of the water (adopted 1933).

## SULFATE OF AMMONIA (COMMERCIAL AMMONIUM SULFATE)

*Sulfate of ammonia (commercial ammonium sulfate)* is a commercial product composed chiefly of ammonium sulfate. It shall contain not less than twenty and five-tenths per cent (20.5%) of nitrogen (adopted 1931).

## SULFATE OF POTASH-MAGNESIA

*Sulfate of potash-magnesia* is a potash salt containing not less than twenty-five per cent (25%) of potash ( $K_2O$ ), nor less than twenty-five per cent (25%) of sulfate of magnesia, and not more than two and one-half per cent (2.5%) of chlorine (adopted 1925).



## SULFATE OF POTASH (COMMERCIAL POTASSIUM SULFATE)

*Sulfate of potash (commercial potassium sulfate)* is a potash salt containing not less than forty-eight per cent (48%) of potash ( $K_2O$ ) chiefly as sulfate, and not more than two and one-half per cent (2.5%) of chlorine (adopted 1929).

## SUPERPHOSPHATE

*Superphosphate* is a commercial phosphate, the phosphoric acid ( $P_2O_5$ ) content of which is due chiefly to monocalcium phosphate. (The grade that shows the available phosphoric acid should always be used as a prefix to the name. Example: 16 per cent superphosphate) (adopted 1939, first action).

## TANKAGE

*Tankage* (without qualification) is the rendered, dried, and ground by-product, largely meat and bone from animals (slaughtered or that have died otherwise) (adopted 1929).

## UNIT OF PLANT FOOD

A *unit of plant food* is twenty (20) pounds, or one per cent (1%) of a ton (adopted 1926).

## UNLEACHED WOOD ASHES

*Unleached wood ashes* are ashes from burned unleached wood that have had no part of their plant food removed and that contain four per cent (4%) or more of water-soluble potash ( $K_2O$ ), (adopted 1928).

## WASTE LIME, BY-PRODUCT LIME

*Waste lime, by-product lime*, is any industrial waste or by-product containing calcium or calcium and magnesium in forms that will neutralize acids. It may be designated by prefixing the name of the industry or process by which it is produced, i.e., gas-house lime, tanners' lime, acetylene lime-waste, lime-kiln ashes, calcium silicate, etc. (adopted 1931).

## INTERPRETATIONS

## ACTIVITY OF WATER-INSOLUBLE NITROGEN IN MIXED FERTILIZERS

*Activity of water-insoluble nitrogen in mixed fertilizers.*—The alkaline and neutral permanganate methods distinguish between the better and the poorer sources of water-insoluble nitrogen, and do not show the percentage availability of the materials. The available nitrogen of any product can be measured only after carefully conducted vegetation experiments.

(a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to three-tenths of one per cent (0.3%) or more of the weight of the material. If a total nitrogen exceeds the minimum guaranty and is accompanied by a low activity of the insoluble nitrogen, the over-run shall be taken into consideration in determining the classification of the water-insoluble nitrogen.

(b) The water-insoluble nitrogen in mixed fertilizers showing an activity below fifty per cent (50%) by the alkaline method and also below eighty per cent (80%) by the neutral method shall be classed as inferior. This necessitates the use of both methods, also the provision as to over-run in (a), before classifying as inferior (adopted 1927).

AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE  
POTASH IS CLAIMED AS SULFATE

*Amount of chlorine permissible in fertilizers in which the potash is claimed as sulfate.*—The *chlorine* in mixed fertilizers in which the potash is claimed as sulfate shall not exceed one-half of one per cent (0.5%) more than what is called for in the minimum potash content based on the definition of sulfate of potash as formulated by the Committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.5 (adopted 1928).

BRAND NAME TO INCLUDE GRADE OF FERTILIZER

*The grade of a fertilizer* should be included with its brand name, and so used by the manufacturer on sacks and in printed literature and by the control official in his reports and publications (adopted 1927).

CYANAMIDE AND UREA NITROGEN

*The nitrogen in cyanamide and urea* is synthetic, non-protein organic nitrogen (adopted 1931).

FERTILIZER FORMULA

*A fertilizer formula* shall express the quantity and grade of the crude stock materials used in making a fertilizer mixture. For example: 800 pounds of 16% superphosphate, 800 pounds of tankage (7.40 nitrogen and 9.15 total phosphoric acid), and 400 pounds of sulfate of potash-magnesia (twenty-six per cent (26%) potash) (adopted 1926).

FINELY GROUND AS APPLIED TO BASIC PHOSPHATE SLAG

*Finely ground* in the definition of basic phosphate slag shall refer to actual size of particles as determined by the use of standard sieves, as follows: seventy per cent (70%) or more shall pass a 100-, and ninety per cent (90%) or more shall pass a 50-mesh sieve (adopted 1927).

THE WORD "LIME" AS APPLIED TO FERTILIZERS

The term "*lime*" shall not be used in the registration, labelling, or guaranteeing of fertilizers or fertilizing materials unless the lime is in a form or forms to neutralize soil acidity (adopted 1935).

NET WEIGHTS

The weights appearing on packages of fertilizer, agricultural lime, and liming materials shall always mean *net weights* (adopted 1932).

ORDER OF TERMS

The *order of terms* in mixed fertilizers shall be nitrogen first, phosphoric acid second, and potash third (adopted 1930).

NAME OF A FERTILIZER MATERIAL USED AS THE BRAND NAME OR PART  
OF THE BRAND NAME OF A MIXED FERTILIZER

When the name of a fertilizer material is used as a part of the brand name of a mixed fertilizer, as for example, blood, bone, or fish, the nitrogen or phosphoric acid shall be derived from or supplied entirely by the material named. When the name of a fertilizer material is used as a brand or as part of a brand and the nitrogen or phosphoric acid is not supplied by the material named, the word "brand" shall follow the name of the materials. Example: "Fish Brand Fertilizer" (adopted 1930).

## STATEMENT OF GUARANTIES

The *statement of guaranties* of mixed fertilizers shall be given in whole numbers (adopted 1930).

## UNIFORMITY IN USE OF TERMS "PHOSPHORIC ACID" AND "POTASH"

As the terms *phosphoric acid* and *potash* are used universally in guaranteeing and in reporting the analyses of fertilizers, it is recommended that the same terms also be used in reporting and discussing the results of analyses of related materials (adopted 1934).

# INDEX

---

- Abrastol, 465  
Acacia, 597  
Acetaldehyde soln, standard, 173  
Acetanilid and acetophenetidin, 559, 561, 563, 564, 633  
Acetate buffer soln, 161  
Acetic acid, and acetylsalicylic acid, combined, 569  
Acetic acid—salt mixture, 215  
Acetic acid soln, 225  
Acetic serum method, added water in milk, 274  
Acetone in spirits, 175  
Acetone-carbon tetrachloride test of fat in cacao products, 205  
Acetophenetidin, 559, 565, 566, 570, 571, 633  
Acetyl value, oils, fats, and waxes, 436  
Acetylsalicylic acid, 567, 569, 570, 571, 633  
Acid, free, in honey, 510  
    mandelic, 610  
    nicotinic, 610  
    tartaric, in fruits and fruit products, 341  
Acid buffer soln, 215  
Acid dyes, 242  
Acid hydrolysis method, fat in baked cereal products, 231  
    fat in macaroni, 235  
Acid number, of boiled linseed oil, 115  
    of dark colored rosin, 82  
    naval stores, 82  
    raw linseed oil, 113  
    volatile oil, spices, 470  
Acid sodium chloride soln, 199  
Acid soln, standard, 22, 650  
Acid-forming or non-acid-forming quality of fertilizers, 37  
Acidity of beer, 152  
    of beverages and concentrates, 146  
    cheese, 301  
    cordials and liqueurs, 182  
    ether extract, eggs and egg products, 314  
    fruits and fruit products, 341  
    grain and stock feeds, 363  
    liquors, tanning materials, 123  
    mayonnaise and salad dressings, 476  
    milk, 269  
    prepared mustard, 475  
    roasted coffee, 196  
    wines, 167, 168, 169  
Acids, common, various strength solns, 656  
    in beer, 152  
        beverages and concentrates, 146  
        cordials and liqueurs, 183  
        crude and refined oils, 436  
        fruits and fruit products, 341, 349  
        industrial water, 545  
        mineral water, 535  
        oils, fats, and waxes, 432, 433, 434  
        spirits, 172, 173  
        tomato products, 522  
        vegetables and vegetable products, 519  
        vinegars, 479, 482  
Aconitine, in aconite root, 581  
    microchemical test, 630  
Acrospire, length of, malt, 156  
Adamkiewicz test, 355  
Aeration method, meats and meat products, 375  
Agar, 597  
Agar agar in fruits and fruit products, 350  
Agricultural dust, 43  
Agricultural liming materials, 41  
Albumin in evaporated milk, 289  
    in flour, 218  
    milk, 271  
Albuminoid nitrogen in grain and stock feed, 356  
Alcohol, amyl, reagent, 604  
    ethyl, and water, percentages by weight corresponding to various percentages by volume at 15.56, 708  
    in almond extract, 327  
    beer, 150  
    beverages and concentrates, 146  
    brandy drops, 508  
    cassia, cinnamon and clove extracts, 328  
    cordials and liqueurs, 181  
    fruits and fruit products, 336  
    ginger extracts, 329  
    lemon and orange extracts, 323  
    peppermint, spearmint, and winter-green extracts, 330  
    sirups used in "brandy drops," 509

- spirits, 172
- vanilla extract, 320
- vinegars, 479
- wines, 164
- various strength solns, 656
- Alcohol extract in spices, 469
- Alcohol precipitate in fruits and fruit products, 340
- Alcohol table for calculating percentages of alcohol by volume at 15.56°C. in mixtures, 708
- Aldehyde-free alcohol, 173
- Aldehydes in cordials and liqueurs, 181
- in lemon and orange extracts, 324
- lemon and orange oils, 326
- spices, 471
- spirits, 173
- Alizarin red indicator, 529
- Alkali, black, in irrigating water, 546
- Alkali salts in soils, 19
- Alkali soln, standard, 25
- Alkalies in industrial water, 545
- various strength solns, 656
- Alkaline salt soln, 574, 588
- Alkaloids, cinchona, 582
- in ergot, 585
- microchemical tests, 629
- Alkanet tincture, 473
- Allihn's gravimetric method, 504
- table, 681
- Almond extract, 327
- Almond paste, 421
- Aloin, 594
- Alpha-naphthol soln, 148
- Alpha-naphthylamine hydrochloride soln, 223
- Alumina in paints, 104
- Aluminum, in baking powders, 190, 191
- in mineral water, 536
- permitted dyes, 257
- plants, 126
- soils, 5
- Aluminum foil, 528
- Amalgam, sodium, 575
- Amaranth in commercial coal tar food colors, 265
- Amido nitrogen, in grain and stock feeds 356
- Amino nitrogen in meat, 382
- in meat extracts, 385
- Aminopyrine, 572
- microchemical test, 633
- Ammonia, in baking powders, 192
- in cottonseed, 446
- meat, 375
- meat extracts, 385
- mineral water, 534
- potable water, 526
- tobacco, 140
- various strength solns, 657
- Ammonia nitrogen, in eggs and egg products, 315
- Ammonia sodium chloride, 265
- Ammonia-cyanide-citrate soln, 397
- Ammoniacal cadmium chloride, 91
- Ammoniacal copper soln, 472
- Ammoniacal silver nitrate soln, microchemical reagent, 629
- Ammoniacal zinc chloride soln, 70
- Ammonium acetate soln, 13, 90, 190, 532, 539
- Ammonium bisulfite soln, 190
- Ammonium chloride soln, 30, 52, 526
- Ammonium citrate soln, 23, 416
- Ammonium dichromate soln, 539
- Ammonium hydroxide for washing, 21
- Ammonium molybdate soln, 133
- Ammonium nitrate soln, 21, 533
- Ammonium oxalate soln, 55
- Ammonium polysulfide soln, 90
- Ammonium sulfate stock and standard solns, 140, 141
- Ammonium thiocyanate soln, 318, 534, 603, 619
- microchemical reagent, 629
- Ampuls, sampling, 559
- Amytal, microchemical test, 634
- Anhydrous sodium carbonate, 652
- Aniline chloride test, 511
- Anise and nutmeg extracts, 330
- Anthranilic acid ester in beverages and concentrates, 147
- Antimony in pigments, 102
- Antimony oxide in paints, 103
- Antipyrine, and caffeine, 573
- microchemical test, 634
- Apomorphine, in tablets, 587
- microchemical test, 630
- Apparent extract of beer, 150
- Apparent viscosity of acidulated flour-water suspension, 227
- Appearance, boiled linseed oil, 115
- varnishes, 109
- Arecoline, microchemical test, 630
- Arsenic, 390
- in baking powders, 192
- Bordeaux mixture, 58, 59, 60
- calcium arsenate, 55
- commercial coal tar food colors, 257
- foods, 390
- gelatin, 386
- insecticides and fungicides, 44, 47
- iron methylarsenate, 616

- iron-arsenate tablets, 615
- lead arsenate, 53, 54
- magnesium arsenate, 56
- mineral water, 542
- Paris green, 52
- plants, 132
- sodium cacodylate, 616
- soils, 10
- zinc arsenite, 56, 65
- Arsenic oxide, in lead arsenate, 54
- Arsenic soln, standard, 390
- Arsenious oxide, in Bordeaux mixture with Paris green, 58
- in calcium arsenate, 55
- lead arsenate, 53
- magnesium arsenate, 56
- Paris green, 52, 53
- zinc arsenite, 56
- Arsenious oxide soln, standard, 44, 73
- Asaprol, 465
- Asbestos, 357, 500
- Ash, in baked products other than bread, 234
- in baking powders, 191
- beer, 152
- beverages and concentrates, 146
- boiled linseed oil, 115
- bread, 231
- butter, 298
- cacao products, 201
- cheese, 301
- commercial coal tar food colors, 256
- confectionery, 507
- cordials and liqueurs, 182
- cream, 287
- dried and malted milk, 292
- evaporated milk, 289
- fish and other marine products, 318
- fruits and fruit products, 337
- gelatin, 387
- grain and stock feed, 354
- honey, 509
- leathers, 116, 117
- lemon and orange extracts, 326
- macaroni, 235
- maple products, 512
- meat, 374
- meat extracts, 384
- milk, 270
- mineral oil-soap emulsions, 63
- naval stores, 83
- nuts and nut products, 419
- phosphated and self-rising flour, 212
- plants, 125
- prepared mustard, 474
- roasted coffee, 194
- rye, oats, corn, buckwheat, etc., 228
- spices, 468
- spirits, 172
- sugars, sirups, and molasses, 487
- sweetened condensed milk, 290
- tea, 198
- tomato products, 521
- vanilla extracts, 322
- vegetables and vegetable products, 518
- vinegars, 478
- wheat flour, 212
- wines, 166
- Ash constituents in vanilla extract, 332
- Assortment of malt, 156
- Atmospheric pressure, correction for variation, turpentine oil, 88
- Atomic weights, international, 655
- Atropine, in tablets, 582
- microchemical test, 630
- Attenuation, real, of beer, 151
- Aurintricarboxylic acid soln, 190
- Babcock method, 272, 287
- Bacteria in tomato products, 523
- Bailey-Andrew method, 198
- Baked products other than bread, 234
- Baking powders and baking chemicals, 186
- Baking test, experimental, for bread, 231
- Barbital, 574
- microchemical test, 634
- Barium, in mineral water, 539
- in paints, 104
- Barium chloride soln, 63
- Bases, available, in calcareous soils, 15
- replaceable, in soils devoid of carbonates, 13
- Basic dyes, 241
- Basic lead acetate soln, standard, 513
- Basic slag, 38
- Baudouin test, 441
- Beaker method, fertilizers, 29
- Beans, hydrocyanic acid formed by hydrolysis of glucosides in, 366
- Beef heart peptic digest broth, 645
- Beer, 150
- Beets, sugar, 515
- Benedict's solns, 305, 499
- Benzaldehyde, in almond extracts, 327
- in beverages and concentrates, 148
- cordials and liqueurs, 184
- Benzocaine, microchemical tests, 630
- Benzoic acid, 456
- in almond extracts, 328

- microchemical tests, 634
- Benzoyl peroxide bleach in flour, 223
- Benzyl morphine, microchemical tests, 630
- Berberine, microchemical test, 630
- Beta-ionone, 332
  - in raspberry flavors, 332
- Beta-ionone-m-nitrobenzhydrazide, optical properties, 333
- Beta-naphthol, 465
- Beverages, malt, 150
  - (non-alcoholic) and concentrates, 146
- Bieber's test, 421
- Bioassay of drugs, 626
- Bismuth compounds in tablets, 617
- Biuret test, 354
- Bleaching powder, 74
- Boiling range of  $\Psi$  cumidine from pon-  
ceau 3R, 263
- Bone, in meat scrap or tankage, 365
  - mechanical analysis, 20
- Boneblack, purified, 170
- Borates, 459
- Borax, standard, 651
- Bordeaux mixtures, 57, 58, 59, 60
- Boric acid, 213
  - and borates, 459
  - in mineral water, 542
- Boric acid soln, standard, 12
- Boron, in fertilizers, 32, 33
  - in soils, 12, 13
- "Brandy drops," alcohol in, 508
- Bread, 228
  - total solids, 229
- Brewers' rice, 163
- Brewing materials, 150
- Brine, 547
- Bromate method for arsenic, 393
- Bromate soln, standard, 44
- Bromide in brine, 548, 549
  - in mineral water, 521
- Bromide-bromate soln, 561, 568, 608, 633
- Bromocresol green indicator, 377
- Bromocresol purple soln, 592
- Bromophenol blue indicator, 49
- Bromothymol blue indicator, 12, 68
- Brucine, microchemical tests, 630
- Buckwheat, 228
- Bushel weight, malt, 155
- Butter, renovated, 293, 300
- Butyro-refractometer reading and indices of refraction, 425
- Cacao bean and its products, 201
- Cacao nibs, shell in, 208
- Cadmium chloride soln, ammoniacal, 91
- Caffeine, 561, 563, 564, 565, 570, 573, 623
  - in roasted coffee, 195
  - tea, 198
  - microchemical test, 630
- Calcareous soils, available bases in, 15
- Calcium, in baking powders, 192
  - in fertilizers, 34
  - fruits and fruit products, 339
  - mineral water, 537
  - permitted dyes, 257
  - pigments, 105
  - plants, 127
  - salt, 550
  - soils, 5, 14, 16
- Calcium acetate soln, 342
- Calcium arsenate, 55
- Calcium chloride soln, acid, 114
- Calcium cyanide, 61
- Calcium gluconate, 617
- Calcium hypochlorite and bleaching flour, 74
- Calcium oxide, in agricultural liming materials, 43
  - in ash, spices, 468
  - calcium arsenate, 55
  - mineral feeds, 365
- Calcium-sodium acetate soln, 342
- Calomel, in calomel ointments, 620
  - in tablets, 620
- Camphor, 575
- Canadian lead number, maple products, 513
- Canned vegetables, microbiological examination, 643
- Capsicum, in ginger extract, 329
- Capsules, sampling, 559
- Caramel in cordials and liqueurs, 182
- Carbon, carbonate, in soils, 2
  - organic, in soils, 3
- Carbon dioxide, correction factors for gasometric determination, 721
  - density table, 719
  - in agricultural liming materials, 43
  - in baking powders, 187, 188
  - beer, 152
  - Bordeaux mixture, 57, 58, 59, 60
  - mineral water, 535
  - pigments, 105
  - self-rising flour, 212
  - sodium hypochlorite solns, 74
- Carbon tetrachloride, purified, 175, 603
- Carbonate in soda lye, 63
- Carbonate carbon, in soils, 2
- Carbonate of lead, basic, in pigments, 96
- Carbonic and bicarbonic acids, in industrial water, 545

- in mineral water, 535
- Carotene, in grain and stock feed, 369
- Cascara sagrada, 595
- Casein, in butter, 298
  - in evaporated milk, 289
  - milk, 270
- Cassia, cinnamon and clove extracts, 328
- Cat-eye method, 626
- Caustic poisons, 80
- Caustic value, agricultural liming materials, 41
- Cereal foods, 211
- Cereal products, baked, 228
- Cheese, 300
- Chenopodium, oil of, 625
- Chicory infusion in coating and glazing substances, 197
- Chinosol, microchemical test, 634
- Chittick's apparatus, 186
- Chloral hydrate, 473
- Chloramine-T, 74
- Chloride, 651
  - in industrial water, 545
  - mineral water, 534
  - potable water, 528
- Chlorides, in ash, of bread, 231
  - of macaroni, 235
  - in beer, 155
    - cheese, 301
    - meat extracts, 384
    - roasted coffee, 195
    - wine, 167
- Chlorine in ash, fruits and fruit products 337
  - in calcium cyanide, 61
  - calcium hypochlorite and bleaching powder, 74
  - Chloramine-T, 74, 75
  - eggs and egg products, 311
  - fat of flour, 222
  - fertilizers, 34
  - fish and other marine products, 318
  - plants, 134
  - sodium and potassium cyanides, 60
  - sodium hypochlorite solns, 73
- Chlorobutanol, 602
- Chloroform in mixtures, 602, 603
- Chlorzinciodide soln, 596
- Cholesterol in mixtures of animal and vegetable fats, 437
- Cinchona alkaloids, 582
- Cinchonidine, 582
  - microchemical tests, 630
- Cinchonine, 582
  - microchemical tests, 630
- Cincophen in presence of salicylates, 607
  - of sodium bicarbonate, 607
  - microchemical test, 634
- Cinnamon, 328
- Citral in lemon and orange extracts, 325
- Citral solns, standard, 325
- Citric acid, in beverages and concentrates, 147
  - in bread, 230
  - cheese, 303
  - cordials and liqueurs, 183
  - dried milk, 292
  - fruits and fruit products, 343
  - milk, 269
  - wines, 169
- Citric acid soln, 534
- Clove extracts, 328
- Coal tar dyes, oil-soluble, 239, 240, 241, 261
- Coating and glazing substances, in roasted coffee, 196
- Cocaine, 584
  - microchemical test, 630
- Cochineal indicator, 25
- Coconut, shredded, 422
- Coconut oil, in cacao butter and fat extracted from milk chocolate, 204
  - silver number for detection, 206
- Cod liver oil in emulsions, 625
- Codeine, 563
  - in elixirs of terpin hydrate, 579
  - tablets, 587
  - microchemical tests, 630
- Coffee and tea, 194
- Cold test for refined wintered salad oils, 440
- Cold-water extract, in ginger, 469
- Collodion ultra filter, 492
- Color, added to eggs, extraction and identification, 316
  - in beer, 150
  - spirits, 179, 180
  - turpentine oil, 84
  - vanilla extract, 323
  - varnishes, 109
  - vinegars, 481
  - of potable water, 525
  - raw linseed oil, 114
  - wort, malt, 160
- Color acid and dye in commercial coal tar food colors, 259
- Color reactions produced on dyed fibers by various reagents, 246, 247
- Color in vinegar removed by fullers' earth, 481
- Color solns, standard, 525
- Color value of vanilla extract, 323
- Coloring matters, in beer, 155



- in beverages and concentrates, 146
  - butter, 299
  - cacao products, 208
  - cheese, 301
  - confectionery, 509
  - cream, 289
  - evaporated milk, 290
  - foods, 239
  - fruits and fruit products, 351
  - green coffee, 194
  - ice cream, 306
  - lemon and orange extracts, 326
  - macaroni, egg noodles, etc, 235
  - meat, 380
  - milk, 279
  - oils, fats, and waxes, 442
  - prepared mustard, 475
  - spirits, 179
  - vanilla extract, 323
  - vegetables and vegetable products, 519
  - vinegars, 483
  - wine, 169, 170
- natural separation, 249
- Colors, artificial, in spirits, 180
- coal tar, in cordials and liqueurs, 182
  - permitted coal tar, separation, 243
- Commercial coal tar food colors, 252
- Composition of mayonnaise and salad dressings, calculation, 476
- Concentrates, 146
- Condensed milk, sweetened, 290
- Condiments, 468
- Conductivity value, maple products, 514
- Confectionery, 507
- Copper, in Bordeaux mixture, 58, 59, 60
  - in fertilizers, 34
  - foods, 414
  - gelatin, 387
  - insecticides and fungicides, 48
  - plants, 130
  - potable water, 533
- Copper carbonate, 57
- Copper nitrate soln, 34
- Copper oxide, in copper carbonate, 57
  - in Paris green, 53
- Copper serum method, added water in milk, 275
- Copper soln, standard, 414, 533
- Copper sulfate, in grain and stockfeeds, 367
- Copper sulfate soln, 130, 138, 498, 504
- Copper-reducing substances by direct inversion, 475
  - in spices, 469
- Cordials, 180
- Corn, 228
  - field, in canned mixtures of field and sweet corn, 519
  - flaked, 162
- Corn grits, 163
- Corn meal, 163
- Corn products, prepared, 162
- Corn-liver medium, 645
- Cotarnine, microchemical tests, 631
- Cottonseed, 443
- Cottonseed oil, 439
- Coumarin in vanilla extract, 320
- Cowles malic acid value, 515
- Cream, 286
- Cream of tartar, in tartrate powders, 188
  - in wines, 169
- Creatin, in meat, 381
  - in meat extracts, 386
- Creatinin in meat extracts, 386
- Crude fiber, in baked products other than bread, 234
  - in bread, 231
  - cacao products, 206
  - flour, 213
  - grain and stock feeds, 357
  - macaroni, 235
  - milk chocolate, 207
  - nuts and nut products, 419
  - plants, 140
  - prepared mustard, 475
  - roasted coffee, 196
  - rye, oats, corn, buckwheat, 228
  - spices, 469
  - tea, 198
- Crude protein, in grain and stock feed, 354
- Cube powder, 64
- Culture media, examination of frozen egg products, 641
  - microbiological examination of canned vegetables, 645
  - thermophilic bacteria in sugar, 642
- Culture technic, thermophilic bacteria in sugar, 642
- Cupric hydroxide, 356
- Cryoscopic method, added water in milk, 275
- Cyanamide, water-insoluble nitrogen in, 28
- Cyanogen, in calcium cyanide, 61
  - in sodium and potassium cyanides, 60
- Cyanogenetic glucosides in feeds, etc., 366
- Dairy products, 269
- Denigès method, modified, methyl al-

- cohol in spirits, 176
- Denigès reagent, 66
- Derris and cube powder, 64
- Devarda method, 27
- Dextrin, in beer, 152
  - in coating and glazing substances, 196
  - fruits and fruit products, 350
  - honey, 510
  - vinegars, 483
  - wines, 170
- Dextro-glucose in nuts and nut products 420
- Dextrose, in eggs and egg products, 312
  - in honey, 510
  - nuts and nut products, 420
  - sugars, sirups, and molasses, 504
- Dextrose tryptone agar, 642
- Diacetylmorphine in tablets, 587
- Diallylbarbituric acid, microchemical test, 634
- Diammonium-citrate soln, 342
- Diastase in honey, 512
- Diastatic power, malt, 160
- Diastatic activity of flour, 225
- Dichlorhydrin, 577
- Di-galacturonic acid in fruits and fruit products, 340
- Diglycol stearate soln, 31
- Dimethylaminobenzaldehyde soln, 585
- Dinitrophenol, 608
  - microchemical test, 634
- Dinitrophenylhydrazine sulfate soln, 614
- Dionine, microchemical tests, 631
- Diphenylamine indicator, 480
- Diphenylamine soln, 141
- Diphenylthiocarbazone, 396, 410, 415
- Dipotassium phosphate, 118
- Disodium phosphate soln, microchemical reagent, 629
- Distillation, turpentine oil, 86
- Distilled liquors, 172
- Dithizone, 396, 410, 415
- Domke's table, 665
- Dried milk and malted milk, 291
- Dried milk products, fat in, 357
- Drugs, 559
  - myotic, 626
- Drying on glass, time of, boiled linseed oil, 115
- Dulcin, 465
- Dust in tea, 197
- Dyes, separation, by immiscible solvents procedure, 241
  - by wool dyeing procedure, 239
- Eberthella typhosa for phenol coefficient, 75
- Edestin in flour, 218
- Egg albumin in coating and glazing substances, 197
- Egg noodles, coloring matter in, 235
- Egg products, frozen, examination, 639
- Eggs and egg products, 308
- Elasticity of varnishes, 109
- Elixirs of terpin hydrate, 579
- Elmslie-Caldwell method, 368
- Emanation method, radioactivity, 552
- Emetine hydrochloride in tablets, 584
- End fermentation of beer, 155
- Ephedra, total alkaloids in, 595
- Ephedrine in inhalants, and tablets, 585
  - microchemical tests, 631
- Ergot, alkaloids in, 585
  - assay of, 627
- Ergotamine tartrate standard soln, 586
- Ergotoxine ethanesulfonate, standard soln, 585
- Esters, in beverages and concentrates, 147
  - in cordials and liqueurs, 183
  - spirits, 173
- Ethanol, 16
- Ether, 604
  - anhydrous, 356
- Ether extract, in confectionery, 507
  - in derris and cube powder, 66
  - eggs and egg products, 314
  - flour, 213
  - grain and stock feeds, 356
  - meat, 375
  - plants, 140
  - prepared mustard, 474
  - spices, 468
- Ether extractives, in commercial coal tar food colors, 258
- Ethoxyl group, microchemical methods, 647
- Ethyl alcohol at 60°F., percentage by volume corresponding to apparent specific gravity at various temperatures, 689
- Ethylhydrocupreine, microchemical test, 631
- Eugenol in volatile oil, spices, 471
- Evaporated milk, 289
- Exchange capacity of soils, 16
- Extract, in beer, 151
  - in corn grit, corn meal, brewers' rice, 163
  - flour, 220
  - malt, 157
  - prepared corn or rice products, 162
  - spirits, 172
  - wines, 165

- Extraction, raw and spent materials, tanning materials, 123
- Extraction apparatus, continuous, 594
- Extracts, in malt, 150
  - in tanning materials, 120
- Facing, tea, 199
- Fat, by acid hydrolysis in eggs and egg products, 310
  - dissolution in acetic acid, critical temperature, 205
  - in baked products other than bread, 234
    - bread, 229
    - butter, 298, 299
    - cacao products, 202, 204
    - cheese, 302
    - corn grits, corn meal, brewers' rice, 163
    - cream, 287
    - dried milk, 292
    - dried milk products, 357
    - evaporated milk, 289
    - flour, 213
    - grain and stock feeds, 356
    - ice cream, 306
    - macaroni, 235
    - malted milk, 292
    - mayonnaise and salad dressings, 476
    - meat, 375
    - meat extracts, 385
    - milk, 272
    - nuts and nut products, 419
    - prepared corn and rice products, 162
    - rye, oats, corn, buckwheat, etc., 228
    - sweetened condensed milk, 290
- Fat acidity, in flour, 213
  - in grain, 363
- Fat number of bread, 229
- Fats, 423
  - in coating and glazing substances, 197
- Fatty acids, in cottonseed, 447
  - preparation of, paints, 94
- Fehling's soln, 161, 498
- Fendler-Stuber method, 195
- Fermentation, real degree in beer, 151
- Ferric ammonium sulfate soln, 567, 603, 619, 625
- Ferric chloride soln, 415
- Ferric chloride test, benzoic acid, 456
  - salicylic acid, 454
- Ferric indicator, 49, 60, 135, 318
- Ferric sulfate soln, 92, 502
- Ferricyanide maltose-sucrose conversion table, 216
- Ferricyanide soln, 215, 225
- Ferrous ammonium sulfate soln, 480
- Ferrous chloride soln, 375
- Ferrous phenanthroline indicator, 502
- Ferrous sulfate, 37
  - in grain and stock feeds, 367
- Ferrous sulfate soln, 343
- Ferrous sulfate-zinc-soda method, nitrate and ammoniacal nitrogen, 27
- Fertilizers, 20
- Field corn in canned mixtures of field and sweet corn, 519
- Filter paper pulp, 130
- Fish and other marine products, 317
- Fish oil and marine oils in presence of vegetable oils and in absence of metallic salts, 442
- Flakes, refined, 163
- Flash point of varnishes, 111
- Flavoring extracts, 320
- Flour, original ash of, in phosphated and self-rising flour, 212
  - in wheat, 211
- Fluorides, in potable water, 529
  - in preservatives and artificial sweeteners, 462
- Fluorine, in insecticides and fungicides, 49
  - in soils, 13
- Folin-Ciocalteu phenol reagent, 281
- Food colors, permitted coal tar, 242
- Foots, raw linseed oil, 114
- Foreign fats containing tristearin in lard, 441
- Formaldehyde, 460
  - in formaldehyde solns, 68
- Formic acid, 466
  - in vinegars, 482
- Fruits and fruit products, 335
- Fuchsin-bisulfite method, 326
- Fuchsin-sulfite soln, 312, 325
- Fuchsin-sulfurous acid soln, 611
- Fuming 38 N sulfuric acid, 88
- Fungicides, 44
- Furfural, 174
  - in cordials and liqueurs, 181
- Furfural soln, standard, 174
- Fusel oil in cordials and liqueurs, 181
  - in spirits, 175
- Galactan in grain and stock feeds, 362
- Galagum, 597
- Gamma ray method, radioactivity, 557
- Gamma undecalactone, in beverages and concentrates, 149
  - in peach and apricot cordials, 183

- Gasoline color value of flour, 224  
Gelatin, 386  
  in coating and glazing substances, 197  
    cottage cheese, 306  
    cream, 289  
    evaporated milk, 290  
    fruits and fruit products, 350  
    meat extracts, 385  
    milk, 279  
Gelatin nitrogen in meat, 381  
Gelatin soln, 123, 199  
Ginger, in ginger extract, 329  
  resin in, 472  
  volatile oil in, 472  
Ginger extract, 329  
Glazing substances in roasted coffee, 196  
Globulin in flour, 218  
Glucose, in beverages and concentrates, 146  
  in confectionery, 507  
  fruits and fruit products, 350  
  honey, 511  
  leathers, 118  
  maple products, 513  
  sugars, sirups, and molasses, 498  
  wines, 166  
Glucosides in beans, hydrocyanic acid  
  formed by hydrolysis of, 366  
Gluten, crude, in flour, 218  
Glutenin in flour, 218  
Glycerol, in beer, 152  
  in cordials and liqueurs, 181  
  eggs and egg products, 312  
  lemon and orange extracts, 324  
  meat extracts, 386  
  shredded coconut, 422  
  vanilla extract, 320  
  vinegars, 480  
  wines, 164, 165  
Glycerol-alcohol ratio, wines, 165  
Glycogen in meat, 378  
Glyoxylic acid test, 355  
Gold chloride soln, microchemical re-  
  agent, 629  
Grade, rosin, 84  
Grain and stock feeds, 353  
Grape juice, added water in, 351  
Grit in poultry and similar feeds, 365  
Grits, corn, 163  
  refined, 163  
Guaiacol, 608  
Gum in wines, 170  
Gum soln, 300  
Gums, identification, 596  
  in mayonnaise and French dressings,  
    477  
  soft curd cheese, 305  
Gunning method, 26, 27  
Gustafson method, 212  
Gutzeit method, 390  
Halogens, in erythrosine, 264  
Halphen test, 439  
Halphen-Hicks test, 95  
Hanus iodine soln, 429  
Hanus method, 429  
Hardness, temporary, of industrial  
  water, 545  
Hehner number, 433  
Hehner test, 460  
Hematin indicator, 123  
Heroine, in tablets, 587  
  microchemical test, 631  
Herzfeld gravimetric methods, 503  
Herzfeld table, 677  
Hexamethylenetetramine in tablets, 576  
Hexylresorcinol, 609  
Hide powder, 121  
Hide substances in leathers, 119  
Hiltner method, 327  
Holtz's cooked meat medium (alka-  
  line), 641  
Homatropine, in tablets, 586  
  microchemical tests, 631  
Honey, 509  
Hopkins-Cole test, 355  
Hortvet cryoscope, 275  
Hydrastine, microchemical tests, 631  
Hydrazine sulfate soln, 147  
Hydrazine sulfate-sodium bromide soln,  
  46  
Hydrazine- $\gamma$ -undecalactone, optical  
  properties, 183  
Hydriodic acid, 608  
Hydrochloric acid, fuming, 144  
  standard, 25  
  standardization, 650  
  various strength solns, 656  
Hydrocyanic acid, formed by hydrol-  
  ysis of glucosides in beans, 366  
  in almond extract, 328  
Hydrogen, replaceable, in calcareous  
  soils, 17  
Hydrogen peroxide soln, 72  
Hydrogen sulfide in mineral water, 534  
Hydrogen-ion concentration, of baked  
  products other than bread, 234  
  of beer, 155  
    bread, 231  
    flour, 213  
    macaroni, 235  
    soils, 17  
Hydroquinone soln, 133  
Hydroxide in soda lye, 63

- Hydroxyquinoline sulfate, microchemical test, 634  
 Hyoscine, microchemical tests, 632  
 Hyoscyamine, microchemical test, 631  
 Hypophosphites in sirups, 617  
  
 Ice cream, 306  
 Identification of natural coloring matters, 250  
   of permitted coal tar food colors, 245  
 Ignited residue, in mineral water, 534  
   in potable water, 525  
 Ignition of soils, loss on, 1  
 Index of refraction, cacao products, 206  
   lemon and orange oils, 326  
   oils, fats, and waxes, 424  
 Indigo carmine soln, 199  
 Indigo soln, 170  
 Industrial water, 545  
 Insecticides and fungicides, 44  
 Insoluble matter, pigments, 101  
 International atomic weights, 655  
 Invert sugar, in honey, 511  
   in sugars, sirups, and molasses, 498  
 Invertase soln, 491, 495  
 Invertase-melibiose soln (bottom yeast extract), 495  
 Iodide, in mineral water, 541  
   in presence of chloride and bromide in brine, 547  
 Iodine, 618  
   in erythrosine, 264  
   iodine ointment, 619  
   mineral mixed feeds, 367  
   plants, 136  
   soils, 6  
 Iodine absorption number, of boiled linseed oil, 115  
   of cacao products, 206  
   oils, fats, and waxes, 429  
   raw linseed oil, 113  
 Iodine monochloride soln, 66  
 Iodine number of fatty acids, paints, 95  
   paprika oil, 472  
 Iodine reaction of beer, 154  
 Iodine reagent, microchemical, 629  
 Iodine soln, 44, 69, 73, 92, 135, 157, 174, 473, 534, 561, 613, 620  
 Iodoform, 619  
 Iodoform gauze, 620  
 Iodoform ointment, 619  
 Ipecac, fluidextract, alkaloids in, 598  
 Irish moss, 597  
 Iron, in baking powders, 191  
   in mineral water, 536  
   permitted dyes, 257  
   plants, 126  
   oxides of, in soils, 5  
   reduced, powder, 141  
 Iron-arsenic tablets, arsenic in, 615  
 Irrigating water, 546  
 Isomeric and similar dyes in amaranth, 263  
 Isopropyl alcohol in spirits, 175  
  
 Jackson-Mathews modification of Nyns' selective method, 504  
   table, 680  
 Jams, soluble solids in, 336  
 Jones modification of Robertson method, 28  
 Jorissen method, 465  
 Jorissen test, 455  
  
 Kaolin, 120, 123  
 Karaya gum, 597  
 Kauri gum soln, standard, 109  
 Kernel paste, 421  
 Kernels, broken, in malt, 156  
 Kerr-Sorber method, modification, 220  
 Ketone in volatile oil in spices, 471  
 Ketones in spirits, 175  
 Kirschner value, 434  
 Kjeldahl method, 26  
 Kjeldahl-Gunning-Arnold method, 26  
 Kleber method, 326  
 Kling method, 342  
 Knapheide-Lamb method, 367  
 Knorr's apparatus, 186  
 Koettstorfer number of oils, fats, and waxes, 432  
 Kraut's reagent, microchemical test, 629  
 Kreis test, 421  
 Krober's table, 686  
  
 Lactose, in cream, 287  
   in evaporated milk, 290  
   milk, 271  
   milk chocolate, 202  
   process cheese, 304  
   sugars, sirups, and molasses, 505  
   sweetened condensed milk, 290  
 Laevo-malic acid in fruits and fruit products, 344  
 Lakes, 239  
 Lane-Eynon method, 498  
   factors for 10-25 ml Soxhlet's soln, 684  
 Langer's method for santonin, modified, 614  
 Leach test, 461  
 Lead, in boiled linseed oil, 115  
   in commercial coal tar food colors, 256  
   foods, 394

- pigments, 102
- potable water, 532
- Lead acetate soln, 343, 344, 633
- “Lead acid,” 90
- Lead arsenate, 53
- Lead chlorofluoride wash soln, 49
- Lead iodide soln, microchemical reagent, 629
- Lead nitrate soln, alkaline, 91
- Lead number, maple products, 513
- in vanilla extract, 321
- Lead oxide, in insecticides and fungicides, 47
- in lead arsenate, 54
- Lead oxide and copper, in Bordeaux mixture, 59
- Lead soln, standard, 395, 532
- Lead thiocyanate, 431
- Lead triethanolamine soln, microchemical reagent, 633
- Leathers, 116
- Leaves, foreign, in tea, 197
- Lemon and orange extracts, 323
- Lemon and orange oils, 324, 326
- Lemon and orange peel color, 326
- Lemon, orange, or lime oil in vegetable and mineral oil, 331
- Leucosin in flour, 218
- Levant worm seed, santonin in, 615
- Levulose, in honey, 510
- in sugars, sirups, and molasses, 504
- Liebermann-Storch test, 95
- Lignin in plants, 143
- Lime, in lime-sulfur solns, 71
- milk of, 480
- Lime oil in vegetable and mineral oils, 331
- Limestone, ground, mechanical analysis, 43
- Lime-sulfur solns, 69
- Liming materials, agricultural, 41
- Lindo-Gladding method, 30
- Linseed oil, boiled, 115
- raw, 112
- Linseed oil paints, white, 90
- Lipoid and lipid phosphoric acid in macaroni, 235
- Lipoid phosphoric acid, in eggs and egg products, 310
- in flour, 220
- Lipoids, in eggs and egg products, 310
- in flour, 219
- Liqueurs, 180
- Liquors, distilled, 172
- in tanning materials, 122
- Lithium in mineral water, 538
- Lithopone in paints, 98
- Litmus indicator, 68
- Liver broth, 642
- Logwood method, 482
- Loss on heating at 105–110°, boiled linseed oil, 115
- raw linseed oil, 114
- Loss on ignition, pigments, 101
- soils, 1
- Macaroni products, 234
- MacMichael viscosimeter, 227
- Magnesia mixture, 21
- microchemical reagent, 633
- Magnesium, in fertilizers, 35
- in fruits and fruit products, 339
- mineral water, 537
- permitted dyes, 257
- pigments, 105
- plants, 128
- salt, 550
- soils, 5, 15, 16
- water-soluble compounds, 36
- Magnesium acetate method, ash in wheat flour, 212
- Magnesium acetate soln, 212
- Magnesium arsenate, 56
- Magnesium nitrate method, sulfur in plants, 133
- Magnesium nitrate soln, 21, 133
- Magnesium oxide in agricultural liming materials, 43
- Magnesium uranyl acetate method for sodium, 130
- Magnesium uranyl acetate soln, 130
- Malic acid, in beverages and concentrates, 147
- in cordials and liqueurs, 183
- fruits and fruit products, 344, 345
- wines, 169
- Malic acid value, maple products, 515
- Malt, 155
- Malt agar, 641
- Malt beverages, sirups, extracts, 150
- Malt extract, 359
- Malted milk, 291
- microscopic identification, 292
- Maltose, in sugars, sirups, and molasses, 505
- Maltose conversion table, 226
- Mandelic acid, 610
- microchemical test, 635
- Manganese, in fertilizers, 37
- in foods, 417
- fruits and fruit products, 338
- grain and stock feeds, 369
- mineral water, 540
- plants, 128
- soils, 6

- Manganous sulfate soln, 531, 540  
Mannite, 12  
Manometric method, CO<sub>2</sub> in beer, 152  
Maple products, 512  
Marine animal oil, in presence of vegetable oils and in absence of metallic salts, 442  
Marjoram, volatile oil in, 472  
Marmalades, soluble solids in, 336  
Marmé's microchemical reagent, 629  
Marsh test, 180  
Martius yellow in naphthol yellow S and its K salt, 265  
Mayer's reagent, 473  
Mayonnaise and salad dressings, 475  
Meal, corn, 163  
Mealiness of malt, 156  
Meat and meat products, 374  
Meat bases, in meat, 381  
Meat extracts, 384  
Meat scrap or tankage, bone in, 365  
Mechanical stirrer method, butter, 293  
Meissl and Hiller's table, 678  
Melting point, of cacao products, 205  
of commercial coal tar food colors, 261  
fats and fatty acids, 426  
Menthol, 599  
Mercuric bromide paper, 390  
Mercuric chloride, 466  
Mercuric chloride soln, microchemical reagent, 629  
Mercuric iodide soln, 271  
Mercuric nitrate soln, 37, 271, 290  
Mercuric sulfate soln, 175  
Mercuric-potassium iodide soln, 473  
Mercurochrome, 621  
Mercurous chloride in tablets, 620  
Mercurous iodide in tablets, 621  
Mercury, in foods, 409  
in mercurial ointment, 623  
ointment of mercuric nitrate, 622  
organic mercurial seed disinfectants, 72  
Mercury soln, standard, 409  
Mercury-thiocyanate soln, 48  
Metals, in beer, 155  
in beverages and concentrates, 146  
commercial coal tar food colors, 256  
confectionery, 509  
foods, 390  
fruit products, 351  
maple products, 513  
meat, 380  
meat extracts, 386  
vegetables and vegetable products, 519  
vinegars, 483  
Methanol, purified, 609  
Metaphenylenediamine hydrochloric oxalic acid soln, 325  
Methenamine, in tablets, 576  
microchemical test, 635  
Methoxy apparatus, 647  
Methoxyl and ethoxyl groups, microchemical methods, 647  
Methyl alcohol, in beer, 155  
in cordials and liqueurs, 181  
lemon and orange extracts, 326  
presence of ethyl alcohol, 611  
spirits, 176  
vanilla extract, 323  
Methyl anthranilate soln, standard, 147  
Methyl orange indicator, 45  
Methyl red indicator, 25, 32, 650  
Methylarsenate, iron, arsenic in, 616  
Methyl salicylate in wintergreen extract, 330  
Methylene blue, 576, 596  
Methylthionine chloride, 576  
Microanalysis of tomato pulp, purée, sauce, and paste, 522  
Microbiological methods, 639  
Microchemical methods, 647  
Microchemical tests for alkaloids, 629  
for synthetics, 633  
Micromethod for reducing sugars, 506  
Microscopic examination of butter, 299  
of nuts and nut products, 421  
spices, 472  
Microscopic identification of malted milk and its flavored products, 292  
Milk, 269  
added water in, determined by freezing-point depression, 717  
dried, 291  
evaporated, 289  
malted, 291  
Milk fat in milk chocolate, 204  
Milk of lime, 480  
Milk protein in cacao products, 201  
Millon's reagent, 80, 217  
Millon's test, 355  
Mineral acidity, leathers, 117  
Mineral acids, free, in vinegars, 482  
Mineral adulterants in ash, of confectionery, 507  
of sugars, sirups and molasses, 488  
Mineral constituents, of confectionery, 507  
of sugars, sirups, and molasses, 487  
Mineral feeds, calcium oxide in, 365  
Mineral oil, and other unsaponifiable matter, test for, in paints, 95  
in turpentine, 88

- Mineral oil-soap emulsions, 62
- Mineral oils, 62
- Mineral water, 534
- Mixed indicator, 37
- Mohler test, modified, 456
- Moisture, in Bordeaux mixtures, 57, 58, 59, 60
  - in butter, 298
  - cacao products, 201
  - calcium arsenate, 55
  - cheese, 301
  - commercial coal tar food colors, 253
  - confectionery, 507
  - corn grits, corn meal, and brewers' rice, 163
  - cottonseed, 445
  - dried and malted milk, 291
  - fertilizers, 20
  - fruits and fruit products, 336
  - gelatin, 386
  - grain and stock feeds, 353
  - ground sample of cottonseed, 445
  - honey, 509
  - insecticides and fungicides, 44
  - lead arsenate, 53
  - leathers, 116
  - macaroni, 235
  - magnesium arsenate, 56
  - malt, 156
  - maple products, 512
  - meat, 374
  - meat extracts, 384
  - mixed or composite pigments, 101
  - nuts and nut products, 419
  - oils, fats, and waxes, 423
  - Paris green, 52
  - plants, 125
  - prepared corn and rice products, 162
  - raw and spent materials, tanning materials, 123
  - roasted coffee, 194
  - rye, oats, corn, buckwheat, etc., 228
  - salt, 549
  - soap, 61
  - soils, 1
  - spices, 468
  - sugars, sirups, and molasses, 484
  - tea, 198
  - wheat flour, 211
  - zinc arsenite, 56
- Molasses, 484
- Mold in malt, 156
- Mold mycelia in butter, 300
- Molds in tomato products, 522
- Molybdate soln, 21, 22
- Molybdenum blue, 347
- Monier-Williams method, 463
- Monobromated camphor in tablets, 575
- Monopotassium phosphate soln, 213
- Monosulfide equivalent, in lime-sulfur solns, 69
- Morphine, 564
  - in sirups, 589
  - tablets, 588
  - microchemical tests, 631
- Morpurgo method, 465
- Munson-Walker general method, 500
- Munson-Walker table, 671
- Mustard, prepared, 474
- Mustard seed, volatile oil in, 472
- Mydriatics and myotics, 626
- Myotic drugs, 626
- Narceine, microchemical tests, 631
- Narcotine, microchemical test, 632
- Natural coloring matters, 249
- Naval stores, 82
- Neocinchophen, microchemical test, 635
- Nessler's reagent, 526
  - modified, 576
- Nessler's soln (Folin), 140
- Neutralizing value, of agricultural liming materials, 41
  - of baking powders, 188
- Nickel acetate soln, ammoniacal, 633
- Nicotine, in tobacco, 141
  - in tobacco and tobacco extract, 64
  - microchemical tests, 632
- Nicotinic acid in tablets and ampuls, 610
- Nitrate nitrogen, in fertilizers, 28
  - in mineral water, 534
  - plants, 141
  - potable water, 527
  - soils, 18
- Nitrate soln, standard, 377, 527
- Nitrates, in fertilizers, 24
  - in industrial water, 545
  - meat, 375
  - meat extracts, 386
  - wines, 170
- Nitric acid, various strength solns, 656
- Nitrite nitrogen in flour, 222
  - in mineral water, 534
  - potable water, 527
- Nitrite soln, standard, 223, 527
- Nitrites, in cured meats, 378
  - in meat, 375
  - meat extracts, 386
  - tablets, 623
- Nitrobenzene in almond extracts, 328
- Nitrogen, in bread, 231
  - in cacao products, 201



- cheese, 301
- commercial coal tar food colors, 267
- confectionery, 507
- cream, 287
- eggs and egg products, 309, 315
- evaporated milk, 289
- fertilizers, 25, 26, 27, 28, 29
- fish and other marine products, 319
- flour, 218, 222
- gelatin, 387
- grain and stock feeds, 356
- ice cream, 306
- leathers, 118
- mayonnaise and salad dressings, 476
- meat and meat products, 375, 380, 381, 382
- meat extracts, 385
- milk, 270
- mineral water, 534
- peppers, 468
- plants, 140, 141
- potable water, 526
- prepared mustard, 475
- soils, 4, 18
- spices, 468
- sugars, sirups, and molasses, 488
- Nitrogen activity, 29
- Nitroglycerin, 578
- Nontannins, in tanning materials, 118, 121, 122
- Non-volatile matter, in varnishes, 109
- Non-volatile vehicle in paints, 93, 94
- Non-volatile water-insoluble matter in commercial coal tar food colors, 254
- Nutmeg extract, 330
- Nuts and nut products, 419
- Oat in flax seed, 448
- Oat hulls in oats and oat feeds, 365
- Oats, 228
- Odor of potable water, 525
- Oil, in anise and nutmeg extracts, 330
  - in cassia, cinnamon and clove extracts, 328
  - cottonseed, 446
  - extracts and toilet preparations, 330
  - mayonnaise and salad dressings, 477
  - mineral oil-soap emulsions, 62
  - peppermint, spearmint, and winter-green extracts, 330
  - spices, 470
- Oil of chenopodium, 625
- Oils of lemon and orange in extracts, 324
- Oils, fats, and waxes, 423
- Ointments of belladonna and stramonium, 599
- Oleomargarine, 300
- Olive oil, tea seed oil in, 440
- 1000 kernel weight of malt, 156
- Opium alkaloids and their derivatives, 587
- Optical rotation, of lemon and orange oils, 326
  - of volatile oil of spices, 470
- Orange II in orange I, 264
- Orange extract, 323
- Orange oil in vegetable and mineral oils, 331
- Organic carbon in soils, 3
- Organic mercurial seed disinfectants, 72
- Ost's soln, 504
- Oxalic acid soln, 169, 342, 346, 530
- Oxalic-sulfuric acid soln, 176
- Oxide, lead, in Bordeaux mixture, 59
- Oxidizing soln, 175
- Oxygen, in potable water, 530, 531
- Paints, 90
- Palm kernel oil in cacao butter and fat extracted from milk chocolate, 204
  - silver number for detection, 206
- Papaverine, microchemical test, 632
- Paprika oil, iodine number, 472
- Paraffin, in confectionery, 508
  - in facing, 199
- Paris green, 52
- Parr's table, 719
- Pasteurization, phosphatase test, 281
- Peanut butter, 420
- Peanut oil, 439
- Peas and canned dried peas, alcohol solids in, 519
- Pectic acid in fruits and fruit products, 340
- Pentosans, in grain and stock feeds, 361
  - in wines, 170
- Peppermint, spearmint, and winter-green extracts, 330
- Peptone in meat, 381
- Perchloric acid method, sodium and potassium, 129
- Percolator, automatic, 581
- Permanganate, alkaline, water-insoluble organic nitrogen distilled from, 29
  - neutral, water-insoluble organic nitrogen soluble in, 29
- Permanganate soln, alkaline, 30
- Permitted coal tar food colors, 242
- Permutit (Folin), 140
- Peronine, microchemical tests, 630, 632
- Petroleum benzin, in leathers, 117

- in naval stores, 83
- roasted coffee, 196
- tea, 198
- Phenacetin, 559, 565, 566
- Phenobarbital, 574
  - microchemical test, 635
- Phenol in caustic poisons, 80
- Phenol coefficient, 75
- Phenol red soln, 593
- Phenoldisulfonic acid soln, 527
- Phenolphthalein, in chocolate preparations, 613
  - in tablets, 571, 612
- Phenolphthalein indicator, 22, 63
- Phenolphthalein soln, 118
- Phenolsulfonates, 623
- Phenylhydrazine bisulfite soln, 190
- Phenylhydrazine hydrochloride tests for formaldehyde, 460, 461
- Phenylhydrazine soln, 184, 326
- Phloroglucin, 361
- Phloroglucinol test, 462
- Phosphatase test for pasteurization, 281
- Phosphate soln, 5, 348
- Phosphoric acid, in ash of roasted coffee, 195
  - of tea, 198
  - in baking powders, 192
    - basic slag, 39
    - beer, 152
    - cordials and liqueurs, 182
    - eggs and egg products, 311
    - fertilizers, 21, 23, 24
    - fruits and fruit products, 347
    - mayonnaise and salad dressings, 476
    - mineral water, 540
    - vinegars, 478
    - wines, 166
- Phosphoric acid-sulfuric acid soln, 313
- Phosphorus, in gelatin, 387
  - in meat, 375
    - meat extracts, 384
    - plants, 133
    - soils, 5, 9
- Phosphotungstic acid soln, microchemical reagent, 633
- Phosphotungstic-phosphomolybdic acid, 321
- Photomicrographs of malted milk, 294
- Physical constants of the 10 % distillate, lemon and orange oils, 327
- Physostigmine, microchemical test, 632
- Phytosterol in mixtures of animal and vegetable fats, 437
- Pigments, in paints, 93, 95, 96, 101
  - used for coloring or facing, 199
- Pigments and lakes, 239
- Pills, sampling, 559
- Pilocarpine, microchemical test, 632
- Pilocarpine hydrochloride in tablets, 589
- Pinene in lemon and orange oils, 327
- Plants, 125
- Platiric chloride method, potassium in plants, 129
- Platinic chloride soln, microchemical reagent, 629
- Platinum soln, 31
- Plato's table, 658
- Podophyllum, 598
- Poisons, caustic, 80
- Polariscopic constants of gelatin, 388
- Polarization, of beer, 152
  - of honey, 509
  - maple products, 513
  - vinegars, 481
- Polenske value, oils, fats, and waxes, 433
- cacao products, 206
- Ponceau 3R, boiling range of  $\Psi$  cumidine from, 263
- Potash, in fertilizers, 30
- Potassium, in baking powders, 192
  - in calcareous soils, 16
  - fruits and fruit products, 337
  - mineral water, 538
  - plants, 129
  - soils, 10, 14
- Potassium and sodium, in soap, 62
  - in soils, 10
- Potassium arsenite soln, 619
- Potassium bromate soln, standard, 393, 615
- Potassium bromide, effervescent, with caffeine, 623
- Potassium bromide soln, 343
- Potassium chloride soln, alcoholic, 50
- Potassium chloride wash soln, 302
- Potassium chromate indicator, 34, 528
- Potassium cyanide, 60
- Potassium dichromate soln, 429, 480, 605
- Potassium dihydrogen phosphate soln, standard, 133
- Potassium ethyl xanthate, 130
- Potassium ferrocyanide soln, standard, 91, 131, 533, 534, 629
- Potassium hydroxide soln, 22, 363, 432, 473, 578, 602, 629
- Potassium iodate soln, standard, 66, 91
- Potassium iodide in grain and stock feeds, 367
- Potassium iodide soln, 54, 90, 135, 225, 390, 396, 531, 547, 568, 608, 609, 629

- Potassium or ammonium thiocyanate soln, standard, 49
- Potassium oxalate soln, 531
- Potassium phthalate soln, acid, 213
- Potassium permanganate soln, 29, 37, 55, 90, 135, 169, 176, 199, 342, 343, 346, 501, 526, 530, 531, 547, 629
- Potassium permanganate soln, standardization, 653
- Potassium sulfate, 135
- Potassium thiocyanate indicator, 92
- Poultry, vitamin D for, 371
- Poultry and similar feeds, grit in, 365
- Power-Chesnut method, 195, 198
- Precipitate, alcohol, in fruits and fruit products, 340
- Preservatives, in beer, 155
- in butter, 299
- cream, 289
- evaporated milk, 290
- fruits and fruit products, 351
- meat, 380
- meat extracts, 386
- milk, 279
- prepared mustard, 475
- vegetables and vegetable products, 519
- vinegars, 483
- wines, 171
- Preservatives and artificial sweeteners, 454
- in beverages and concentrates, 146
- Preserves, soluble solids in, 336
- Procaine, 590
- Procaine hydrochloride, microchemical test, 632
- Protein, in baked products other than bread, 234
- in beer, 152
- bread, 231
- dried and malted milk, 292
- flour, 217
- fruits and fruit products, 341
- grain and stock feeds (qualitative tests), 354
- macaroni, 235
- nuts and nut products, 419
- prepared mustard, 475
- rye, oats, corn, buckwheat, etc., 228
- sweetened condensed milk, 290
- tea, 198
- wines, 170
- Protein-nitrogen, precipitable by 40% alcohol in flour, 219
- in macaroni, 235
- Proteins, milk, in cacao products, 201
- Protease, in meat, 381
- in meat extracts, 385
- Pyramidon, 572
- Pyrethrin I, in mineral oil, 67
- in pyrethrum powder, 66
- Pyrethrin II in derris and cube powders, 67
- Pyrethrum extracts in mineral oil, 67
- Pyrethrum powder, 66
- Pyridium, 613
- microchemical test, 635
- Quince gum, 597
- Quinidine, 582
- microchemical test, 632
- Quinine, 563, 564, 582
- microchemical test, 632
- Quinine and strychnine, separation, 592
- Quisumbing-Thomas method, 138
- table, 685
- Racemate method, total tartaric acid, fruits and fruit products, 342
- Rachitic ration, basic, 371
- Radioactivity, 552
- table, 726
- Radon method, radioactivity, 552
- Raffinose in sugars, sirups, and molasses, 495
- Raw and spent materials, tanning materials, 123
- Reaction of certain natural coloring matters to common reagents, 251
- Reaction values of soils, 18
- Reducing substances, in vinegars, 479
- Reducing sugars, in fruits and fruit products, 350
- in maple products, 513
- sugars, sirups, and molasses, 506
- vegetables and vegetable products, 518
- Refractive index of turpentine oil, 86
- of volatile oil of spices, 470
- Reichert-Meissl value, 433
- of cacao products, 206
- Renovated butter, 300
- Resin, in ginger, 472
- Resin oil in oils, fats, and waxes, 439
- Resorcinol test, 511
- Rice, brewers', 163
- flaked, 162
- Rice hulls in rice bran, 364
- Rice products, prepared, 162
- Robertson method, 28
- Rochelle salt soln, acidified, 582
- Roeese-Gottlieb method, confectionery, 508

- cream, 272, 287
- ice cream, 306
- milk, 272
- Rosin, 82
  - dark colored, acid number, 82
  - grading, 84
  - in naval stores, 83
  - paints, 95
- Rotene in derris and cube powder, 64
- Ruthenium red soln, 596
- Rye, 228
- Rye flour in wheat flour, detection, 225
- Saccharimeters, temperature corrections to readings, 664
- Saccharin, 458
- Saccharometric indication of beer, 150
- Sage, volatile oil in, 472
- Salad dressings, 475
- Salicylates, total, 568
- Salicylic acid, 454
  - free, 567
  - in presence of other phenols, 572
  - microchemical test, 635
  - standard soln, 567
- Salol, 566, 571
- Salt, 549
  - in butter, 298
  - fish and other marine products, 318
  - grain and stock feeds, 364
  - meat, 374
  - nuts and nut products, 420
  - prepared mustard, 474
  - method of reporting results, 550
- Salt acetate soln, 262
- Salt soln, 314
- Sand, 390
  - in plants, 125
  - tomato products, 521
- Sanglé-Ferrière method, 465
- Santonin in mixtures, 614, 615
- Saponification number, boiled linseed oil, 115
  - cacao products, 206
  - naval stores, 82
  - oils, fats, and waxes, 432
  - raw linseed oil, 113
- Scales method, 499
- Schiff's reagent, modified, 176
- Schultze's mixture, 473
- Schweitzer's reagent, 472
- Scopolamine, microchemical test, 632
- Scrubber trap for nitroglycerin, 578
- Sediment test, milk, 280
- Seed disinfectants, organic mercurial, 72
- Seeds, foreign, in malt, 156
- Selenium, in foods, 417
  - in plants, 137
  - soils, 11
- Sesame oil, 441
- Sewage, 40
- Shell in cacao nibs, 208
- Silica, in industrial water, 545
  - in mineral water, 535
  - plants, 125
  - soils, 4
- Silicotungstic acid soln, 64
  - microchemical reagent, 633
- Silver in drugs, 624
- Silver ammonium hydroxide, 377
- Silver carbonate, 480
- Silver compounds, ionizable, detection and estimation, 624
- Silver nitrate soln, 34, 49, 60, 135, 318, 528, 540, 602, 633
- Silver number of coconut and palm kernel oils, 206
- Silver proteinate, 624
- Silver sulfate soln, standard, 527
- Sinibaldi method, 465
- Sirups, 484
  - malt, 150
- Soap, in insecticides, 61, 63
- Soda lye, 63
- Soda-lead, 61
- Sodium, in baking powders, 192
  - in chloramine-T, 75
  - mineral water, 538
  - plants, 129
  - soap, 62
  - soils, 10
  - metallic, 604
- Sodium acetate, in brilliant blue FCF, 225
- Sodium acetate soln, 415, 466, 608
- Sodium alizarin sulfonate soln, 13, 51, 348
- Sodium- $\alpha$ -naphthol-2-sulfonate soln, 147
- Sodium amalgam, 575
- Sodium benzoate soln, microchemical reagent, 629
- Sodium bicarbonate, separation from acetanilid, 562
- Sodium bicarbonate soln, 148, 595
- Sodium bisulfite soln, 174
- Sodium borate, 651
- Sodium cacodylate, arsenic in, 616
- Sodium carbonate in erythrosine, 264
- Sodium carbonate fusion, soils, 4
- Sodium carbonate soln, 147, 526, 546, 629
- Sodium carbonate-sucrose soln, 37
- Sodium chloride, in commercial coal tar food colors, 254

- in macaroni, 235
- tomato products, 521
- vegetables and vegetable products, 518
- Sodium cobaltinitrite, 14
- Sodium ethylate, 314
- Sodium fluoride soln, 529
- Sodium hydroxide, various strength solns, 656
- Sodium hydroxide soln, 12, 22, 25, 30, 32, 44, 50, 68, 73, 90, 113, 214, 310, 347, 357, 433, 528, 531, 541, 547, 568, 608, 649
- Sodium hypochlorite solns, 73
- Sodium iodide in erythrosine, 264
- Sodium nitrate soln, standard, 376
- Sodium nitrite soln, 147, 541, 633
- Sodium oleate soln, 396
- Sodium peroxide method, sulfur in plants, 132
- Sodium polysulfide soln, 396
- Sodium salicylate, 564
- Sodium sulfate, in commercial coal tar food colors, 255
- Sodium sulfite soln, 133, 548, 613
- Sodium thiosulfate soln, 34, 46, 92, 174, 225, 367, 396, 414, 429, 531, 547
- Sodium tungstate, 215
- Sodium and potassium cyanides, 60
- Soil reaction values, 18
- Soils, 1
- Solids, in baked products other than bread, 234
- in beverages and concentrates, 146
- bread, 229
- canned peas and canned dried peas, 519
- cordials and liqueurs, 181
- cream, 287
- eggs and egg products, 308
- evaporated milk, 289
- fruits and fruit products, 336
- ginger extracts, 329
- industrial water, 545
- jams, 336
- leathers, 118
- lemon and orange extracts, 325
- liquors, tanning materials, 122
- macaroni, 235
- maple products, 512
- marmalades, 336
- mayonnaise and salad dressings, 475
- milk, 270
- mineral water, 534
- oysters and scallops, 318
- potable water, 525
- prepared mustard, 474
- preserves, 336
- roasted coffee, 194
- sugars, sirups, and molasses, 485
- sweetened condensed milk, 290
- tanning materials, 120, 121
- tomato products, 520
- vanilla extract, 322
- vegetables and vegetable products, 518
- vinegars, 478
- wheat flour, 211
- wines, 166
- Solutions, standard, 649
- Somogyi's dextrose-thiosulfate equivalents, 506
- Sørensen method, 383
- Sour serum method, water in milk, 274
- Soxhlet's modification of Fehling's soln, 498
- Soya flour in uncooked cereal products, 228
- Sparteine, microchemical test, 632
- Spearmint extract, 330
- Specific gravity, of beer, 150
- of beverages, and concentrates, 146
- boiled linseed oil, 115
- cordials and liqueurs, 181
- lemon and orange extracts, 323
- lemon and orange oils, 326
- milk, 269
- mineral water, 534
- oils, fats, and waxes, 423
- paints, 96
- raw linseed oil, 112
- spirits, 172
- tomato products, 520
- turpentine oil, 84
- vanilla extract, 320
- vinegars, 478
- volatile oil of spices, 470
- wines, 164
- Spices in vinegars, 483
- Spices and other condiments, 468
- Spirits, 172
- Spirits of turpentine, 84
- Spores in tomato products, 523
- Standard phenol soln, 80
- Standard solns, 649
- Stannous chloride soln, 265, 390
- Staphylococcus aureus for phenol coefficient, 78
- Starch, in baking powders, 189
- in beer, 154
- cacao products, 207
- chopped meat, sausage, deviled meat, 378

- confectionery, 507
- flour, 221
- fruits and fruit products, 350
- grain and stock feeds, 359
- peanut butter, 420
- roasted coffee, 196
- spices, 469
- Starch gum, 597
- Starch indicator, 34, 44, 91, 135
- Starch iodate paper, 92
- Starch iodide paper, 265
- Starch iodide soln, 547
- Starch soln, 90, 161, 225, 396
- Starch-potassium iodide soln, 215
- Stems in tea, 197
- Stock feeds, 353
- Stovaine, microchemical test, 632
- Stramonium ointment, 599
- Strontium in mineral water, 537
- Strychnine in tablets and liquid preparations, 591
- microchemical tests, 632
- Strychnine and quinine, separation, 592
- Sucrol, 465
- Sucrose, by double dilution method, 497
- in beverages and concentrates, 146
- cacao products, 201
- confectionery, 507
- cordials and liqueurs, 182
- eggs and egg products, 312
- fruits and fruit products, 349
- grain and stock feeds, 359
- honey, 510
- lemon and orange extracts, 326
- maple products, 513
- mayonnaise and salad dressings, 476
- plants, 139
- sugar beets, 515
- sugar solns, Abbé or refractometric readings, at other temperatures than 20° C, 669
- Refractive indices at 20° C, 667
- Zeiss immersion refractometer readings at 20° C, 670
- sugars, sirups, and molasses, 488, 491, 495, 497
- sweetened condensed milk, 290
- vanilla extract, 322
- vegetables and vegetable products, 519
- wines, 166
- Sucrose solns at 20° C, Domke's table of apparent specific gravity of, 665
- Sugar in bread, 231
- in coating and glazing substances, 196
- meat, 379
- meat extracts, 386
- nuts and nut products, 420
- vinegars, 479
- thermophilic bacteria in, 642
- Sugar analysis, rules of International Commission for Unifying methods, 488
- Sugar beets, 515
- Sugar solns, ° Brix, specific gravity, and ° Baumé, 658
- Sugars, in baked products other than bread, 234
- in beer, 152
- beverages and concentrates, 146
- flour, 215
- fruits and fruit products, 350
- grain and stock feeds, 358
- honey, 510
- mayonnaise and salad dressings, 475
- plants, 138
- roasted coffee, 196
- spirits, 176
- tanning materials, 121
- tomato products, 521
- wines, 166
- Sugars and sugar products, 484
- Sulfanilamide, 614
- microchemical test, 635
- Sulfanilic acid soln, 222, 527
- Sulfate, in salt, 550
- in pigments, 106
- lime-sulfur solns, 71
- Sulfate of lead in pigments, 97
- Sulfates in vinegars, 482
- Sulfide soln, 25
- Sulfide sulfur, in lime-sulfur solns, 70
- in pigments, 106
- Sulfite agar, 642, 646
- Sulfite cellulose in tanning materials, 122
- Sulfite-cellulose soln, 122
- Sulfite-fuchsin soln, 173
- Sulfonal and trional, 581
- Sulfonated dyes, lower, in commercial coal tar food colors, 262
- Sulfonphthalein indicator, 213
- Sulfur in commercial coal tar food colors, 259
- in fruits and fruit products, 337
- lime-sulfur solns, 69
- pigments, 106
- plants, 132
- soils, 8
- Sulfur dioxide in beer, 154
- in gelatin, 388
- pigments, 107
- Sulfur dioxide soln, 254

- Sulfuric acid, in baking powders, 192  
  in industrial water, 545  
  mineral water, 537  
  wines, 167  
  fuming 38 N, 88  
  standard, 25  
  standardization, 652  
  various strength solns, 656  
Sulfurous acid, 463  
  in wines, 170  
Suspended matter, potable water, 526  
Sweetened condensed milk, 290  
Sweeteners in beer, 155  
Sweeteners, artificial, 454  
  in vegetables and vegetable products, 519  
Sweetening substitutes in fruits and fruit products, 351  
Synthetics, microchemical tests, 633
- Tables, reference, 654  
Tablets, sampling, 559  
Tankage, bone in, 365  
  mechanical analysis, 20  
Tannin, in cloves and allspice, 469  
  in leathers, 118, 119  
  tanning materials, 121  
  tea, 199  
  wines, 169  
Tanning materials, 120  
Tartaric acid, in baking powders, 188  
  in beverages and concentrates, 147  
  cheese, 302  
  cordials and liqueurs, 183  
  fruits and fruit products, 341  
  vinegars, 482  
  wines, 169  
Tartaric acid soln, 302  
Tartrate soln, 138, 498, 504  
Tartrates in vinegars, 482  
Tartrazine in commercial coal tar food colors, 265  
Tea, 194, 197  
Tea seed in olive oil, 440  
Temperature correction for specific gravity of oils, 423  
Terpin hydrate in elixirs, 579  
Tertiary butyl alcohol in spirits, 175  
Tetrachlorethylene in mixtures, 604  
Theobromine, in theobromine calcium, 593  
  microchemical test, 632  
Theophylline, 593  
  microchemical test, 632  
Thermophilic bacteria in sugar, 642  
Thiocyanogen number of fats and oils, 431
- Thiosulfate soln, standard, 25, 54, 215, 501  
Thiosulfate sulfur, in lime-sulfur solns, 70  
Thinner, in paints, 93  
Thorium nitrate soln, 51, 529  
Thujone, in cordials and liqueurs, 184  
Thymol, 600  
  in antiseptics, 600  
Thymol blue indicator soln, 313  
Tin in foods, 412  
  pure metallic, 396  
Titanium, oxides of, in soils, 5  
Titanium pigment in paints, 99  
Titanium trichloride soln, 259, 613, 625  
Titer test for oils, fats, and waxes, 427  
Tobacco, ammonia in, 140  
  free nicotine in, 141  
Tobacco and tobacco extract, 64  
Toluene distillation method, 353  
Toluene moisture method, 20  
Toluene-insoluble material in naval stores, 83  
Tomato products, 520  
Toughness of varnishes, 109  
Tragacanth, volatile acidity of, 601  
Tragacanth gum, 597  
Travers' method (modified), 50  
Tribasic lead acetate soln, 344  
Triethanolamine, microchemical test, 635  
Trimethylamine soln, 178  
Trional, 581  
Tristearin, detection in lard, 441  
Turpentine oil, 84  
Turbidity of potable water, 525
- Un sulfonated residue in mineral oils, 62, 63  
Unsaponifiable residue, in flour, 220  
  in linseed oil, 113, 115  
  macaroni, 235  
  oils, fats, and waxes, 438  
Uranyl indicator for zinc titration, 91
- Vanilla extract, 320  
Vanilla resins in vanilla extract, 322  
Vanillin in vanilla extract, 320, 321  
Van Slyke method, 382  
Varnishes, 90  
  oleo-resinous, 109  
Veal infusion agar, 641  
Vegetable tanned leather, 116  
Vegetables, canned, microbiological examination, 643  
Vegetables and vegetable products, 518  
Villavecchia test, modified, 441

- Vinegars, 478  
Viscosity, of acidulated flour-water suspension, 227  
of varnishes, 112  
Vitamin D for poultry, 371, 524  
Vitamin D milk, 282, 524  
Vitamins, 524  
Volatile acids, in beer, 152  
in beverages and concentrates, 147  
fruits and fruit products, 341  
ginger, 472  
marjoram and sage, 472  
mustard seed, 472  
naval stores, 83  
oils, fats, and waxes, 423  
spices, 469  
Volatile thinner, paints, 93  
Wagner's microchemical reagent, 629  
Wash soln, 178  
Water, in ice cream, 287  
in milk, 274  
mineral oil-soap emulsions, 62  
paints, 92  
sausage, 374  
potable, 525  
Water extract in tea, 198  
Water test, varnishes, 112  
Water-insoluble matter in commercial coal tar food colors, 253  
Water-insoluble solids in fruits and fruit products, 336  
Water-soluble material in leathers, 117  
Waters, and brine, reporting results, 543  
brine and salt, 525  
Waxes, 423  
in coating and glazing substances, 197  
Waxy substances in facing, 199  
Wein's table, maltose, 679  
Wheat flour, 211  
Wichmann method, 321  
Wijs iodine soln, 430  
Wijs method, 113, 430  
Wijs soln, 90  
Wilson receiving flask, 331  
Wines, 164  
Wintergreen extract, 330  
Winton lead number, 514  
Winton method, 321  
Wort, color of, malt, 160  
Xanthoproteic test, 356  
Xylene distillation method, moisture in soap, 61  
Xylenol, method, 377  
Yeasts, in tomato products, 523  
Yohimbine, microchemical test, 632  
Zeisel methoxyl apparatus, 647  
Zinc, 390  
in foods, 415  
gelatin, 388  
pigments, 104  
plants, 131  
potable water, 534  
Zinc arsenite, 56  
Zinc chloride soln, microchemical reagent, 629  
Zinc oxide, in insecticides and fungicides, 48  
in paints, 98  
zinc arsenite, 56  
Zinc potassium iodide soln, microchemical reagent, 629  
Zinc pyridine soln, microchemical reagent, 633  
Zinc soln, standard, 415, 534  
Zinc sulfate soln, 91, 131